

What to do to help in India

Mrs Indira Gandhi's assassination is unlikely to see a retreat for support for Indian science. But in the turmoil, it may be neglected. People elsewhere should do more than they think possible to help.

THOSE who believe that the scientific community is in some sense international had better pay special attention to the plight in which Indian scientists will now find themselves. The assassination last week of Mrs Indira Gandhi will be, for them, more than a mere political calamity. The scientific enterprise in India, whatever its faults may be, owes its growth since independence from the British in 1947 to the policies deliberately followed first by Jawaharlal Nehru, the first Prime Minister of India, and then by his daughter. Their objectives were open and straightforward. Understanding the natural world would in due course be a source of economic prosperity, social improvement and the banishment of superstition.

It is ironical that the most striking proof so far of the wisdom of this policy, the new prosperity of the cereal-growing regions of northern India, may have helped to stir the fears of the fundamentalist Sikhs that their emotional hold on their followers would be attenuated, thus provoking the fortification of the Golden Temple at Amritsar, the Army's counter-move and last week's hail of bullets. There is no risk that Mrs Gandhi's son and her successor will draw the wrong lesson from this sad sequence of events, and conclude that things have been moving too quickly. The danger is, rather, that the new government will be so overwhelmed with the problems abounding in the wake of the assassination (not to mention the election soon to be held) that it will have neither the energy nor the resources for an enterprise whose benefits will be delayed beyond tomorrow.

That is why this is a time for recognizing once more the immediate social benefits to contemporary India of its scientific establishment. This huge country, which is in reality six or seven countries, suffers from a curious sense of lacking a cultural identity. Regional diversity is only part of the trouble. Mrs Gandhi may have been right in complaining (see page 93) that outsiders have no perception of how the long occupation by the British impoverished the country.

For at least a century, the intellectual life of India has been profoundly influenced by the universities, originally created on the pattern of the University of London in 1857. Over the years, the system has generated a distinguished roll of intellectual heroes, whose names are widely known and who are generally respected. The difficulty, for India, is that the achievements of these heroes are in a foreign idiom, one that may even be held to have been imposed from the outside. Part of the Nehru-Gandhi commitment to science and technology may be explained by the need to make a distinctive mark. That, certainly, is why centres of excellence are scattered through India. And the commitment had already gone so far, before independence, that the brief Janata government of the 1970s found it impractical to aim at what it foolishly supposed to be simpler goals. Having come so far, India cannot go back — which does not imply that chairmen of atomic energy commissions should not be sanskrit scholars on the side, or that the distinctive Indian sense of kinship (with places as well as people) need diminish.

The scientific enterprise also has much to offer in the solution of other problems rife in contemporary India. Since the time of Homi Bhabha, in the 1950s, the government of India has been dedicated to the belief that scientific enterprises would help to teach the country as a whole more productive ways of working. The Atomic Energy Commission was the spearhead of this move-

ment, and has now spawned a host of other institutions in the same or a similar mould — from the space programme to superb medium-sized laboratories such as the Physical Research Laboratory at Ahmadabad. The commission's plans for building nuclear power stations may have been less realistic than it had been hoping, but it has offered India a lesson now being widely learned — that difficult technical tasks can be carried through by people who are resolute enough, and meticulous, that it is not necessary in the process that each qualified person's hands should be held up by a small army of assistants and that it is possible for very large projects to be carried through without the corruption endemic in much of India's commercial life. The enterprise is a model of how to get good things done.

In principle, the scientific enterprise is also a model for the cure of that common if less corrosive social disease in India — the bureaucracy that seems to flourish everywhere. The difficulty is inescapable in a democracy where procedures derive from law, but need not be as much an impediment as it is in India. The difficulty has become a serious problem chiefly because those who operate the procedures derive power and therefore pleasure from the processing of pieces of paper that determine what other people should do. The scientific enterprise, however, recognizes that it cannot flourish when hamstrung in this way, and has in many places been able to make a dash for freedom. That is another model that the new government needs to have to hand.

Successive Indian governments' commitments to the goal of self-reliance (potential self-sufficiency) is more controversial. The late Lord Blackett found himself in hot water when he declared, when delivering the first Nehru Memorial Lecture in New Delhi, that it would be prudent for India to take fuller advantage of the willingness of people and governments elsewhere to help with the innovation then so plainly necessary. The shock is still remembered. There is something in the view, which Mrs Gandhi held, that a country as potentially powerful as India cannot perpetually be beholden to others. But may this not be a time when a softening of the line would be wise, if only to allow those who wish to help have something they can do?

The problem of the poor is different. Nobody denies the opinion general in India that a person is not less deserving of respect because he or she happens to be illiterate. But can such a person be as dispassionate, and as solid a member of society, as India's ambitions for democracy would require, if he is more likely to be hurt by infectious diseases or natural catastrophes? India may be lucky not have driven bulldozers through its villages in some rash attempt to make the country modern, but it is hard to believe that the enrichment of the poor would be calamitous. Ironically, there are many in the technical community of India anxious to do more, as Mrs Gandhi, to help with the rural poverty. Is this a time to catalyse their energies?

Governments swept into office in tragic circumstances such as those in India now are of two kinds — those who change nothing for fear that any change will bring more tragedy and those who recognize change to be a way of leavening a sense of shock by a sense of hope. Pandit Nehru and then Mrs Gandhi were persuaded that their scientific enterprise would indeed engender a better India. Their successor would be readily applauded if he were to ask for still greater efforts from those with whom rests the future of Indian agriculture, health and industry.



Outside India, there is also much that can be done. A critical account of science in India (*Nature* 308, 581; 1984) provoked a stream of entertaining correspondence with a single theme — how much more Indian scientists (at home and overseas) might do to help India if they had a chance. May not this be a time for them to try a little harder? More to the point, may it not also be a time when the colleagues of Indian scientists, in India and elsewhere, should exert themselves more energetically to help? Nobody would pretend activities such as these would quell communal unrest, making it safe for Sikhs to walk the streets again. But both parts of the now divided community will need to know that their divisions will eventually be submerged in a wider unity. Go give a lecture, send a book, write a letter, is too trite for anything but a personal exhortation. Governments in the West can do more, and should, to help the enterprise along. An intelligent woman's death requires no less. □

Europe in the sky

Europe is preparing for adventures beyond the atmosphere, but without much thought.

ONCE upon a time (in the early 1960s) Great Britain was a disappointed space power (as the saying goes), with a design for a rocket launcher (called *Bluestreak*) whose development it could not afford to finish. So what more natural, Great Britain said to its European neighbours, than that everybody should pool resources and set up a common organization (eventually called ELDO, for European Launcher Development Organization) to rescue something from the frustrated enterprise? Everybody also agreed that space would be expensive, and so agreed that there should be a common effort on research (whence ESRO, for European Space Research Organization). After many adventures (and a great deal of poor management provoked by indecision), the governments of Western Europe decided that one organization would be enough (called ESA, for European Space Agency). But all this time, Great Britain knew it could get what it wanted more cheaply by making a deal with its friends across the Atlantic (or with NASA, for National Aeronautics and Space Administration, an agency of the US government). But then the time came when the US government was also short of money, wanting to spend every penny it could find on other kinds of rockets, so that it started asking its partners to dig in their pockets as well as its own. But poor Great Britain was by this time really poor and had nothing at all to spend. And so NASA flew off with its rich new friends — France, West Germany and Italy. Poor Great Britain had no choice but to set up a committee to ponder what to do.

This is how legend will encapsulate the developments last week, in Britain and on the mainland, in European policy on space. The cause of everybody's difficulty is NASA's request for paying participants in the manned space platform. France, West Germany and Italy have it in mind to reply with a counter-proposal, one likely to satisfy NASA's need to show to Congress that people elsewhere are prepared to help. Britain's trouble is not merely that the Science and Engineering Research Council (SERC) which would normally have an important say in such a project, is just now wondering which parts of basic science should be abandoned, but that the government as a whole is having to decide from which of two or three ministries to extract a further £1,000 million of spending money. The mainland, long since impatient, will be exasperated.

The moral in the tale is, however, simple. The Richmond committee (see page 92) has advised SERC to throw its lot in with ESA because its terms of reference were too narrow to allow a more adventurous proposal. What happens to British space policy in the next ten years is, however, beyond the competence of grant-making agencies. The government, not the council, should now decide what should be done, preferably in concert with its European partners, say what it intends to do and then stick with its decision. Making such a decision would no doubt be hard, but not making one will have even more serious consequences. □

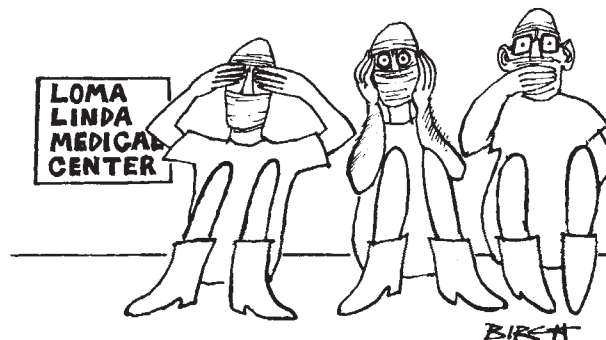
Grandstand medicine

Baboon's hearts are not taboo for people, but medicine which is not essential is wrong.

THE baboon-heart transplant performed on a critically ill infant at Loma Linda Medical Center in California is disquieting. As a medical milestone, to be sure, it is a remarkable accomplishment. Conceivably, it will someday even be a genuine treatment for the 1 in 3,000 infants born with hypoplastic left heart syndrome, the uniformly fatal defect from which the infant in California suffered. Moreover, it is important that the causes of disquiet should not be misrepresented. The need to sacrifice a healthy baboon so that a human infant might live is simply not the ethical dilemma that animal rightists, with an unfailing nose for the sensational, are pretending. A society that overwhelmingly approves of eating animals (other than people) would be even more astonishingly illogical than usual in its reaction to special cases if it were to deny the propriety of using a lower animal to save a human life. And the mere fact that an animal organ is being inserted in a human is an ethical issue only to those whose residue of mythological sentiment overpowers their capacity for rational thought; is there strictly a difference between the use of pig insulin and baboon hearts to make good deficiencies in human beings?

The serious difficulty over the operation carried out in California is that it may have catered to the researchers' needs first and to the patient's only second. Over the past seven years, Dr Leonard Bailey, the surgeon heading the transplant team, has performed 150 such transplants on animals. There can be no doubt that the team was more than eager to try out its technique on a human. Dr Bailey's sincerity and his commitment to helping infants born with this and other tragic birth defects is above reproach. But it is fair to ask if this was the time to extend what Dr Bailey himself calls a "highly experimental" operation to humans.

It is not reassuring that the Loma Linda hospital did not even check with the California Regional Organ Procurement Agency to find out if a suitable human heart was available. (As it turned out, one was; it is not known whether it would have proved



compatible.) It is less reassuring that hospital spokesmen first said they had not known that a human heart was available, and then claimed that in any event it would have taken five days to match the tissues of donor and recipient. (According to researchers at Stanford University, a major centre for heart transplants, the procedure takes only eight hours.) Bailey then acknowledged that a human donor had not been sought because, simply, the purpose of his experiment was to see if the baboon heart would work.

Bailey has been given approval and has been promised support from Loma Linda to perform five more baboon-heart transplants on human subjects. One can only hope that before the next operation, the hospital will agree to release its protocols for the operation and copies of the informed consent forms that parents are asked to sign; so far the hospital has refused to do so. This is not nitpicking; although the research is privately supported, Loma Linda should feel an obligation to the scientific community if not to the public as a whole to provide reassurance. Otherwise suspicions will arise that its researchers are playing to the grandstands. □

French research

New minister plans for selectivity and flexibility

Paris

M. HUBERT Curien, newly-appointed minister of research and technology in France, is preparing to make French science policy really work.

For three years, French scientists have enjoyed close attention, encouragement and even cash from a government which saw — and still sees — science as the nation's salvation. But the economic results have been disappointing, and major government programmes in biotechnology and electronics, for example, have been criticized as substantial failures.

Publicly, Curien does not agree. ("It's difficult for a minister to admit failures", he said in his Paris office last week.) But within the ministry, he is making a careful assessment of the "*programmes mobiles*" set in train by his predecessor, Jean-Pierre Chevènement, in 1982.

Selectivity is the watchword. The trouble with the early dreams was their breadth, all of biotechnology, for example, or all of electronics. But France has only 50 million people and, more tellingly, a history of success in narrow rather than broad technologies such as: nuclear power, space and the high-speed trains. Curien's task must now be to focus the programmes more narrowly.

Curien was previously president of the French space agency (CNES) and of the European Space Agency (ESA), and he recognizes the difficulty. "It is much easier to make good central policy in things like space, where there are few customers and, when you have half-a-dozen programmes, you've covered all the field."

Curien says that while the biotechnology programme did not achieve everything, it created a number of small companies and developed a view of what French industry needed to do. So Curien now has to choose. So far, he has picked agriculture and food, where many of the French traditional industries (such as wine and cheese) are both of great importance to the French economy and in very poor contact with French science. This must change "very rapidly" if the industry is to remain competitive.

In electronics, similarly, there must be rationalization and selection, he says. He cites as promising fields software, where France is already strong, robotics ("we've made some advances") and computer hardware where, says Curien, we should expect a new French product in the mid-range between a microcomputer and a minicomputer.

French researchers should also find themselves with more computers next year, according to Curien. He claims to have cut

the Gordian knot which effectively forbade French laboratories to buy foreign computers while the French industry struggled to catch up. This policy has led to serious under-equipment particularly in nuclear physics groups. But from next year, says Curien, groups will be free to buy computers which are "essential" for their laboratories if they will not be available "in the coming years" in France.

Curien is also seeking more money for industrial research, both from industry itself and from banks. "There is still a large gap between the [1982] plans and reality." France will have to make a "big effort" to catch up with Japan or even West Germany. In the big companies it is a question of rate; in the small, that they do no research at all. The big companies can be influenced from Paris but Curien says the government encouragement of regional science policy is beginning to have important effects on the smaller enterprises. Curien is also consulting the banks about legal obstacles to raising risk capital. In France, a person once bankrupt is forbidden from ever running a business again.

The minister also plans to raise the political temperature of science again next year (cooler now than in the Chevènement years) by staging a major debate on French

research and development in the National Assembly. The topic, ostensibly, will be that of what is to be done in 1985 when Chevènement's 1982 law on the orientation and support of French science comes to the end of its term. "It may be a new law, it may not", says Curien noncommittally. One possible theme is a linkage between the ministry of research and technology and the Administration du Plan, the French ministry that makes long-term "plans" for France with a considerable degree of independence from the president or prime minister — and a sometimes equal independence from effect.

Another key issue for Curien is the old chestnut of finding jobs for young researchers, or the ageing of the research community. So far France has not met the 4.5 per cent recruitment target of the 1982 law but has kept up a respectable 3 per cent; but even this cannot go on, says Curien. "We cannot solve this problem by creating new jobs every year. We must have a real policy." In 10 years the problem will change as the "recruitment bulge" retires but the bulge comes at different times in different agencies so that retirement and recruitment must be managed.

Curien says that making the scientists civil servants (in a new employment contract to be published soon) "will ease the situation". The point is that the new contract will give researchers "a parachute" — a guarantee of their old jobs back — if they shift into something new (such as industry). Curien hopes this will cause movement and, no doubt, that in the end not too many parachutes will have to be used.

Robert Walgate

West German research

Industry favoured by budget

Aachen

THE budget of the West German Ministry for Research and Technology, which forms 70 per cent of the total government civil research budget, is to be increased by 3 per cent in 1985. This is more than the growth in the national budget (1.2 per cent), and reflects the attempt of the government to keep abreast of new technological development.

The distribution of the budget of DM 7,250 million clearly demonstrates the government's intention to promote industrial research efforts. Whereas support for the reactor in Hamm has been reduced by 6.1 per cent, industry will be given increased support, both directly through a 28.5 per cent increase in research subsidies, and indirectly, through a reduction in taxes and financial support for research projects.

Particular encouragement is given to the employment of young scientists by companies with fewer than 3,000 employees and a yearly turnover of less than DM 300 million. Sixty per cent of the salaries of new research staff will be paid

for 15 months when these conditions are fulfilled. Exchange of younger scientific personnel from industrial research laboratories to academic laboratories will also be supported to stimulate transfer of information between the sectors.

In particular, the redistributed budget is designed to assist the foundation of more new technology-oriented companies for which an increase of financial support of nearly 150 per cent is foreseen. Directly supported research institutes, which absorb more than one-third of the whole budget, are expecting an increase of 5.3 per cent. Furthermore, Dr Heinz Riesenhuber, the Minister for Research and Technology, stresses the need for adequate research into the consequences of new technological developments. In the face of the effect of pollution on the forests in Germany, efforts are to be made to evaluate a wide range of developments, from the use of methanol as a fuel to the value of the electronics industry in improving the employment market. A 122 per cent increase in funds for such studies is anticipated.

Jurgen Neffe

NIH budget

Reagan vetoes busybody bill

Washington

EFFORTS by the US Congress to create a new arthritis institute and a new nursing institute at the National Institutes of Health (NIH) have been blocked. President Reagan last week vetoed a controversial NIH reauthorization bill passed in the last few days of the 98th Congress which, besides creating the new institutes, would have required the appointment of disease prevention directors and a formalized NIH policy on matters as different as the use of experimental animals and investigation of scientific fraud. Because Congress is not in session, the so-called "pocket veto" cannot be overridden. In his memorandum of disapproval, the president said the bill would create expensive new organizations, institutionalize over-specific research and place undue constraints on executive branch authorities.

The bill, which had received wide bipartisan support in Congress, was the culmination of a two-year effort by Representative Henry Waxman (Democrat, Los Angeles) to introduce more congressional control over NIH. Waxman last week described as "callous and insensitive" the president's veto of a bill that would "improve our ability to find a cure for cancer, arthritis and stroke".

The reaction at NIH, however, is likely to be one of unmitigated relief. While some of the bill's provisions would merely have added statutory authority to guidelines already in use, others were seen as unwarranted meddling in NIH's internal affairs. The creation of the new institutes was particularly opposed, on the grounds that it would introduce new administrative costs to little effect. The president's veto does not threaten existing NIH programmes, which can continue to operate (as they have since 1980) under the general authority of the Secretary of Health and Human Services to conduct and support research. Congressional aides said after last week's veto was announced that there is still widespread interest in reauthorizing NIH and that the veto will dissuade a future Congress from moving to pass similar legislation. Some predicted that parts of the now-dead bill will be resurrected in a new legislation next year.

The compromise version of the bill that eventually emerged from a House-Senate conference represented a considerable watering-down of Waxman's original proposals. The requirements placed upon institutes to undertake specified kinds of research would nevertheless have had an immediate impact in some instances. The bill would also have established a plethora of statutory bodies to oversee research in different areas. Present regulations on *in utero* fetal research would have been encoded in law, as would the requirement for peer review of research grants.

The bill had included a provision to set up a Congressional Ethics Advisory Commission to study human genetic engineering and fetal research, to have been modelled on Congress's Office of Technology Assessment. Despite last week's veto, preparations to establish an advisory committee of some sort are going ahead: it is not yet clear what sorts of legal authority are required for different functions of an advisory committee.

One of the few parts of the authorization bill that NIH is sorry to lose is that relating to use of experimental animals. The requirements were almost identical with new guidelines already being implemented by

NIH, but a legal obligation to follow the guidelines would have allowed NIH to defend itself more strongly against organizations protesting about the use of animals in research.

The separate appropriations bill for NIH also passed recently by Congress is still waiting for the president's signature. Congress awarded NIH \$5,100 million for fiscal year 1985, an increase of 14 per cent on the previous year, considerably more than the administration's request. There is however little reason for the president to veto the appropriations bill, because a back-up appropriation for NIH included in the continuing resolution provides an identical sum. For the time being, at least, it seems that NIH has succeeded in both having its cake and eating it.

Tim Beardsley

Molecular science

Japan's next target — biology

Tokyo

A THREE-YEAR project to develop in Japan a whole new set of automated tools for molecular biology and cancer diagnosis is about to be launched. The Science and Technology Agency (STA) hopes for rapid government approval for a plan to apply Japan's skills in electronics and precision manufacturing in this field.

Overall expenditure for the project is expected to run to around ¥2,000 million (\$8 million) with ¥618 million (\$2.6 million) allowed for in the first year. The money will come from the special co-ordination funds of the Council for Science and Technology, Japan's highest science policy-making body.

Announcement of the project comes just as Prime Minister Yasuhiro Nakasone has agreed to give top priority to cancer research in next year's budget. Since he gave his approval to a ten-year programme for cancer control last year, several of the science ministers have announced new plans — and new funds — for cancer research (see *Nature* 310, 264; 1984). Now, Nakasone seems to have given tacit approval to an overall 17.8 per cent increase in funds for the next fiscal year.

The overall chief of the new STA project will be Dr Toyozo Terashima, deputy director of the National Institute of Radiological Sciences. There are two main themes: technologies that can directly facilitate research into the molecular biology of cancer, and technologies of use in the diagnosis and therapy of cancer.

In the first theme, attempts will be made to speed up several routine but time-consuming procedures of the molecular biology laboratory. A good part of the research will be performed by industrial companies, in cooperation with university and government research institutes.

Watch-makers Seiko will continue development of an automatic DNA sequencing machine (*Nature* 307, 193;

1984), while film-makers Fuji Photo will improve their new gels for DNA electrophoresis. The chain of events may then be completed by electronics giant Hitachi, trying to develop computerized equipment to read the gel electrophoresis pattern.

Meanwhile, a large chemical manufacturer, Toya Soda, will develop equipment to extract specific DNA fragments from a DNA mixture and tackle the difficult task of building an automatic amino acid sequence analyser. And Japan Electron Optics Laboratory will attempt to develop technology to analyse the three-dimensional structure of protein-DNA complexes using electron beams.

Another goal is to find new ways of introducing DNA into cells: earlier this year, a group at STA's Institute of Physical and Chemical Research (RIKEN) developed a system for opening tiny holes in cell walls using a very brief laser pulse. In the one-second interval before the hole closes spontaneously, DNA from the surrounding medium can enter the cell. Further new systems, using both viruses and very short high-power electric pulses, are now to be tried out by a pharmaceutical company, Dai-ichi Seiyaku. RIKEN and the Tokyo Metropolitan Institute of Medical Sciences will also try new techniques to extract and purify very small amounts of protein produced with the proliferation of cancer cells. New assay cell culture systems for oncogenes will be developed at the Tokyo Medical and Dental University.

In the second part of the project, RIKEN and the National Institute of Radiological Services will commence work on the analysis and synthesis of glycoproteins located on the surface of cancer cells, and the Electrotechnical Laboratory on computer tomography for the detection of cancer. Finally, the huge Sumitomo Electric Company is to seek ways of speeding up production of monoclonal antibodies.

Alun Anderson

Genetic manipulation

NIH concede part of Rifkin suit

Washington

THE US government has quietly conceded a major claim in the lawsuit brought by anti-genetic-engineering activist Jeremy Rifkin to halt field trials of recombinant microorganisms.

Last May (see *Nature* 24 May, p.296), US District Judge John Sirica placed a preliminary injunction on all such experiments supported by the federal government, and in particular an experiment planned by Steven Lindow of the University of California at Berkeley to test the ability of genetically-engineered bacteria to protect potato plants against frost damage. Sirica ruled that in approving that experiment, the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH) had failed to satisfy two legal requirements: it had not conducted a formal environmental assessment of the experiment, and it had not prepared a much lengthier environmental impact statement on the entire programme of environmental release of recombinant DNA.

In an appeal filed with the US Circuit Court of Appeals in Washington, the government has now indicated that it will prepare an environmental assessment of the Lindow experiment and any others involving release to the environment. The government is continuing to appeal against Sirica's finding that what is called a "programmatic impact statement" is required. In a separate appeal, the University of Cali-

fornia is challenging both requirements.

According to Bernard Talbot, deputy director of the National Institute of Allergy and Infectious Diseases, the NIH institute that contains RAC, attorneys of the Department of Justice had pressed NIH to drop its opposition to the environmental assessment requirement, feeling that the government's case would be much stronger if it were limited to the issue of the future programme. The government argues that there is in fact no "programme" of release to the environment, and that the proposed experiments are so widely different that a case-by-case approach is the only one possible.

The Office of Recombinant DNA Activities at NIH has already begun to compile an environmental assessment of the Lindow experiment, but NIH officials make it clear that they consider the exercise meaningless, fulfilling a formal legal requirement without adding anything of substance. The Lindow experiment was debated at two public meetings of RAC, and was revised in response to concern expressed by the committee. NIH appear to be hedging their bets by agreeing to go ahead with the environmental assessments at this stage. And with the University of California continuing to press the full appeal, the government clearly had little to lose.

Although Sirica's injunction specifically exempted experimental proposals from commercial companies — which have been

What, no uranium?

Canberra

AUSTRALIAN exports of uranium oxide to France are likely to stop for at least two years. The Australian Minister for Resources and Energy, Senator Walsh, announced last month that the first eight shipments of yellowcake that were to have been sent to the French power company Electricité de France under a contract with the Nabarlek uranium producer, Queensland Mines Limited, will be deferred because of the continuation of nuclear testing at the French underground site at Mururoa Atoll, in the south central Pacific.

This is in line with the Australian government's ban on uranium sales to France confirmed during the Labor Party's national conference in Canberra in July, when a vote to continue mining and export of uranium was carried (see *Nature* 2 August, p.354). The first shipment (due in October) would have been 100 tonnes of yellowcake valued at \$A6 million. The Australian government has agreed to purchase the first eight contracted shipments at the original price, but Queensland Mines is obliged to repurchase the uranium "if circumstances permit". This is interpreted to mean if and when the French stop testing nuclear weapons in the Pacific.

Jeffrey Sellar

submitting them to RAC for approval on a voluntary basis only — NIH director James Wyngaarden has ordered that environmental assessments be carried out for these as well. At its June meeting, RAC approved two commercial proposals from Advanced Genetic Sciences (AGS) — almost identical to the Lindow experiment. Once the Lindow document is completed, it should be a quick matter to prepare for the AGS proposal, and that experiment should be clear to go ahead by next spring.

The appeals court is scheduled to hear the case on 5 December. Although the appeal is technically on the preliminary injunction only, for practical purposes the court's decision will decide the entire case. If the appeals court upholds Sirica's decision, the government will probably concede the case rather than let it drag on to a near-certain outcome.

Rifkin was notably unsuccessful on another front last week, RAC unanimously rejected his proposal to ban all transfers of genetic material from one mammalian species to the germline of another. The committee noted the benefits that such research holds for the study of cancer and other diseases. Researchers at the University of Pennsylvania have already successfully inserted the gene coding for human growth hormone into mice embryos, and are planning similar experiments on sheep; RAC also noted the possible benefits that such research would bring in more efficient food production.

Stephen Budiansky

Gloomy outlook for young neuroscientists

Los Angeles

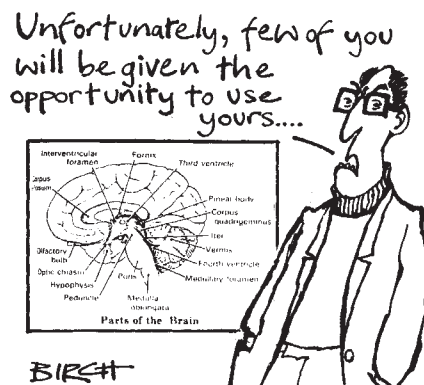
AS the US Society for Neuroscience held its 14th annual meeting in Anaheim from 10 to 15 October, there was talk in the corridors of great frustrations and dashed expectations.

But the subject did not revolve around difficult research questions in neuroscience. Rather, it applied to scores of young scientists who are dropping out of the discipline because financial support and job prospects are woefully inadequate.

Neuroscientists are not the only group to complain about support for the students they train. But, argued Dr Dominick Purpura, last year's society president, because neuroscience is reaping great intellectual rewards, it is attracting many of the brightest and best students in US universities.

Once trained, the young neuroscientist is faced with "incredible competition", Dr Purpura said. "Many universities will take on a young scientist for one or two years, whereupon the scientist must provide his or her own financial support. But the young are competing against established people for limited funds. There is so much compe-

tion that people are disheartened and drop out." The job placement centre at the meeting interviewed 1,200 candidates for fewer than 250 jobs, mostly postdoctoral appointments without long-term security.



The National Institutes of Health and the National Science Foundation together support 85 per cent of research in biology and biomedicine, Dr Purpura said. "They are \$50 to \$100 million short in grants that might allow young neuroscientists to get a start."

Sandra Blakeslee

Japanese technology

MITI seeks friends from abroad

Tokyo

JAPAN'S Ministry of International Trade and Industry (MITI) last week took the remarkable step of inviting foreign representatives to attend a joint meeting of two of its key councils concerned with the planning of research and development projects.

A major new bill to promote high technology research is in preparation (see *Nature* 310, 614; 1984), and MITI seems anxious to sound foreign opinion before presenting the bill to the *Diet*. What remains unclear is whether the meeting will turn out to have been a sop to those feeling threatened by MITI's new plans or a first step in an initiative to encourage international research cooperation. For the foreigners, at least, the hope was that this would be the first of many meetings.

The two councils, the Industrial Technology Council and the Industrial Structure Council, between them play the major role in deciding the thrust of MITI's big research and development projects. All too often, these projects have been fiercely criticized by foreign governments for "targeting", or for aiming to use the combined strength of government and industry to achieve Japanese technological domination in some highly profitable area.

Recent projects have evoked strong reactions worldwide. Those under way at the moment include the fifth generation computer project (which has frightened many advanced nations into launching their own "artificial intelligence" programmes); an optoelectronics project designed to rid optical fibre circuits of relay electronics through the use of optical switching; the massive sunshine and moonlight projects intended to exploit new energy sources (geothermal, solar and biomass) and to find new ways of conserving and storing energy; a host of projects on improving industrial performance through use of robots, flexible manufacturing systems equipped with lasers and computerized sewing and garment assembly machines; and long-term projects aimed at new ceramics and superlattice semiconductors.

The first MITI project that really evoked foreign discontent, however, was the VLSI (very large scale integrated circuit) project that ran from 1976 to 1980 and which involved 100 researchers from the seven large computer manufacturers, MITI's own electrotechnical laboratory and the telecommunications monopoly NTT's electrocommunications laboratory. Virtually all aspects of the production of microchips were investigated, seven hundred new inventions were patented and within a year of the end of the project, Japanese manufacturers had seized 70 per cent of the world market in 64K random access memories (rams) and were leading world

research in the production of 256K rams and 1 megabit microchips.

US electronics companies sent reeling by this assault on their markets vigorously protested that the pooling of competing companies' research resources was unfair — particularly as their own anti-trust laws would have made a similar strategy impossible. Japanese companies on the other hand show little respect for the value of the MITI project, and instead attribute success in the world microchip market not to a mythical "Japan Inc." but to the fierce competition among themselves to produce the finished item as cheaply as possible.

Since that time, there have been regular demands from the United States to allow foreigners to act as witnesses, or even participants, on Japanese government research councils. While this may seem unreasonable interference in another government's deliberations, the United States says it seeks only a clear understanding of new legislation before it can cause industrial hostilities. In Japan that means seeing the legislation before it is submitted to the *Diet* which can hardly be considered a major forum for debate when more than

eighty per cent of bills pass without any amendment whatsoever. (The general procedure is that a consensus is worked out behind the scenes before a bill is ever presented.)

In the event, the representatives invited to the MITI councils' meetings — from the United States, United Kingdom, West Germany and France — were from their respective Chambers of Commerce rather than from embassies. MITI itself made no specific proposals, although suggestions have been made that foreign companies operating in Japan may be allowed to use the new industrial technology centre that MITI wants to set up, and that exchanges of personnel may be encouraged in areas in which Japan now leads the world. Foreign representatives were however, given a chance to say how international cooperation might be brought about.

Other attempts by Japan to encourage major international research projects have so far yielded little fruit. The series of proposals made at the Williamsburg summit, such as that for the development of new robots to work in dangerous environments, may have hung fire because they tried to involve all the summit nations. Smaller scale projects arrived at through bilateral agreement may prove more successful.

Alun Anderson

Britain in space

Strong links with Europe urged

BRITISH scientists and grant-making agencies are urged to play a fuller part in the work of the European Space Agency (ESA) in the report of a committee to consider future policy. The committee, under Sir Mark Richmond, the vice-chancellor of the University of Manchester, also says that its parent, the Science and Engineering Research Council, should take the initiative in persuading government departments to set up a strong coordinating body for British space research.

Among academics working in the field, the overwhelming reaction is relief that the Richmond committee has not advocated a retreat from space research. The same interests complain that the committee has not openly advocated some way of financing space research other than by subventions from the academic research budget. No doubt the committee would have been more explicit if it had some inkling of what the British government would be prepared to spend (if anything).

The origin of the committee, reflected in the arguments of the report now published, is the financial pressure of British space research in recent years. The committee points out that British government spending in the civil field is £80 million a year, compared with £200 million a year in West Germany and £300 million a year in France (the main sponsor of the Ariane launcher).

The committee notes that British scientists have in the past been able to sustain

their research by means of bilateral agreements, chiefly with the United States, largely on the strength of good science and competence in the design and construction of instruments. But this period, the committee says, is drawing to an end, partly because British scientists are no longer outstanding among Europeans, partly because they have less to offer in cash as well as kind, partly because of some recent failures of instruments built for other people's satellites (such as some of the detectors of the EXOSAT satellite) and partly because of a change of US policy, now slanted towards collaboration with ESA wherever possible.

The Richmond committee acknowledges the relatively high cost of international collaboration but says that if British academic science does not intend to abandon space altogether, it should participate more fully in ESA. On particular points, the committee recommends a transfer of the Science and Engineering Research Council's interests in remote sensing by satellite to the Natural Environment Research Council and the encouragement of British industry to interest itself in microgravity experiments.

The Richmond report has not been formally adopted by the Science and Engineering Research Council. The council is probably as embarrassed as the committee by uncertainty about the government's financial intentions. □

European space

Another Franco-German deal

Paris

FRANCE and West Germany have reached "a very good agreement" on collaboration in space and on the European response to the invitation to participate in the US space station, French science minister Hubert Curien said last week.

No figures were discussed between Curien and his German opposite number, Heinz Reisenhuber, when they met in Bonn on Wednesday, but there was agreement in principle that the French should join in with the "Columbus" space station project proposed by Germany and Italy, while Germany would take a share in the HM60 cryogenic motor under development in France for the Ariane-5 space launcher.

Thus it seems that a continental, Franco-German-Italian plan has emerged for a space programme for the European industry in the 1990s, with the other potential major partner, Britain, left on the sidelines. Curien was "sure", however, that Britain would participate "though I'm not certain at what percentage". British government sources have said that the nominal 2 per cent that Britain took in Ariane would certainly be too small.

But whatever share Britain takes, it is clear that European mainland is now calling the tune. No British proposals have emerged that could be traded against Columbus and Ariane-5 to provide a package more suited to Britain. British Aerospace's HOTOL concept of an air-breathing, runway-using hybrid between the space shuttle and Concorde is too distant a prospect to be of use in negotiations, and the proposed free-flying robotic platform may be too like parts of Columbus.

Meanwhile, Europe's space scientists are attempting a defence of their territory, the mandatory science budget of the European Space Agency. In principle, the space station is part of ESA's optional programme. Microgravity and Earth resources research are also optional, coming under the transportation and applications budgets, but the "traditional" space sciences of high-energy astronomy, magnetospheric research and planetary sciences are mandatory to ESA members. Space scientists in this traditional area fear an erosion of mandatory budget — whereas in fact it has long been claimed that it should be increased.

Just by chance, the ESA space science directorate has now come forward with a rational plan as to how that budget should grow. Called the "Horizon 2000" study, it was prepared from 77 responses from the larger space science institutes around Europe to a request for realistic projects for the 1990s. Whittled down by an ESA committee, it is a proposal for a 50 per cent growth in real terms over eight years. The

programme would be based on four cornerstones:

- A solar terrestrial programme (space plasmas)
- A mission to asteroids and comets including return of material to Earth
- A high throughput X-ray mission for spectroscopic studies at 0.1–20 keV
- A high-throughput heterodyne spectroscopy mission to cover the last remaining unexplored region of the electromagnetic spectrum.

Mrs Indira Gandhi

A sharp-witted democrat

MRS Indira Gandhi, killed last week, had the sharpest wit. I met her at the end of February when her attempt to negotiate a settlement of the Sikh rebellion with community leaders from the Punjab had collapsed. It would have been forgivable that she should have cancelled a meeting with a visiting journalist interested only in her expectations of Indian science. Perhaps she welcomed a chance to talk about something completely different.

Disconcertingly, this did not prevent her from reading and signing her mail while keeping command of the conversation. Occasionally, members of her staff would creep into her office with messages and



would be told what to do by signals, a movement of the hand or eye that did not interfere with the flow of words.

There was an awkward moment at the beginning, when it seemed as if the Prime Minister thought that *Nature* is a magazine devoted to environmental causes; she launched on an eloquent speech about the beauty of the Indian landscape that threatened to use up the 45 minutes available. But, when prompted, she changed the course of her conversation without batting an eyelid.

Quite apart from Mrs Gandhi's belief (taken over from her father) in the importance of research and development in India, she exuded affection for the field and its practitioners — "my scientists" she called them. Her gratitude to people like Professor M.G.K. Menon (who has held

Such missions would not be in competition with United States or Japanese projects, it is claimed, but could be part of a balanced world programme of space research.

The Horizon 2000 study has been very well received in West Germany and Switzerland, where both countries are willing to provide the 7 per cent budget increase a year it implies, but other ESA members are proving more reluctant. M. Curien, for France, says he might find 5 per cent "at a great stretch", and a clutch of other states are talking of a mere 3 per cent. Britain has nothing to say just yet.

Robert Walgate

almost every senior scientific post in the government that there is) was evident. So was her pleasure at having as colleagues people such as Dr T. Koshoo, now Secretary at the Department of the Environment, whom she had inveigled to Delhi over a period of two years.

Mrs Gandhi was astoundingly knowledgeable about the vast programme of research her government mounted. Her willingness to visit laboratories and construction sites was renowned. One consequence was the high morale of those who worked there.

With some of her problems, Mrs Gandhi was plainly impatient. University administrations had let authority slip from their grasp, she implied; they should be more vigorous, more like her perhaps. But then, visitors from overseas should beware of the trap of applying in India the standards conventional in the West. The real wisdom of India rests with the village peasants, hardly any of whom can hope that their children will find their way to a university. It was easy to see how other Indian politicians, allies and critics alike, acknowledged that her electoral strength lay in the villages.

So how to reconcile the poverty with India's ambitions in high technology — nuclear power, space technology, electronics and so on? Mrs Gandhi was eloquent on the theme that a sovereign state as powerful as India could not allow itself to remain in thrall to the West. "Look at what they did to us over the fuel for Tarapur", a reference to the ham-handed administration of the US Anti-Proliferation Act towards the end of the Carter presidency.

Mrs Gandhi's demeanour throughout this conversation was in my experience extraordinary. She was direct to the point of being unguarded; no doubt she had heard it all before, but she gave no hint of calculating what to say. She was practised at tormenting the British about colonial rule, yet able to talk as a woman of the world. She gave no sign of trading on her political power. Manners aside, her courtesy was her frankness.

John Maddox

Agricultural research

International centres under stress

Washington

THE Consultative Group on International Agricultural Research (CGIAR) meets in Washington this week to decide how the 13 research centres under its control are to respond to continued financial uncertainty. Exchange rate losses caused by the increased value of the dollar, and the growing tendency of donors to support specific programmes judged to bring local benefits rather than backing core research, have meant that the heady expansion of the 1970s has been all but forgotten.

The centres had a total nominal budget last year of \$180 million, but the failure of some donors to meet in full their commitments to the centres has, for several years running, left programmes high and dry. For 1985, the CGIAR technical advisory committee is requesting a total budget of between \$187 million and \$199 million, an increase of 7-14 per cent on the current year, but actual reductions in 1985 are not impossible. The traditional supporters of CGIAR — of which the United States is the largest — are under internal pressure to restrict multilateral aid in favour of support for bilateral projects, whereas the newer donors have not yet been able to make a substantial impact. The World Bank remains the donor of last resort, paying the bills that others have failed to meet.

The Rockefeller Foundation, which founded the first of the agricultural research centres (for breeding wheat, in Mexico) and the Ford Foundation, a substantial sponsor from its early days, have been reducing their contributions to the centres in recent years. Rockefeller is turning instead to centres outside the CGIAR system and is to announce a major initiative in plant cellular and molecular biology before the end of the year, probably based in Mexico. The budget will be in the region of \$5 million a year, but already some are asking whether the Rockefeller Foundation will be able to recruit to Mexico enough high-calibre scientists to justify this outlay.

Despite the financial uncertainty, CGIAR appears confident that most of the centres are still doing a good job. But there is frustration over the plight of some African centres, which have to work with often under-developed national programmes. Local interests seek to direct research efforts towards strictly local problems, an approach which CGIAR feels to be unproductive in the long term. The centres which have historically been the most effective seem to be those with a strictly defined mandate, whereas attempts at some centres to work on a number of different local problems are held to have been less productive.

The International Centre for Agricultural Research in Dry Areas, in Syria, and

the International Livestock Centre for Africa, in Ethiopia, are likely to come under particular scrutiny this week, and there may be attempts to tighten up their programmes. Particular attention will also be paid to the West Africa Rice Development Association (WARDA) in Liberia, which has been under heavy criticism. The future of WARDA within the CGIAR system appears still to be open to doubt, despite some attempts at reform by the WARDA management.

CGIAR has at various times been criticized for advancing the careers of scientists

rather than the fortunes of the developing countries it is intended to benefit. The criticism is apparently taken seriously, to judge from the number of introspective studies by CGIAR now in progress. Should its centres, for example, try to stand in place of national agricultural development programmes where these are seen to be inadequate? There was some agreement in preliminary discussions last week that centres in different parts of the world should be ready to adapt their strategies to local circumstances despite the dangers. There was also an agreement to adopt a plan to foster cooperation between the various institutes, including a joint training programme in farming systems research.

Tim Beardsley

Early success a problem for CGIAR

Washington

THE international research centres supported by CGIAR stem from a crop improvement programme undertaken in the 1940s by the Mexican government and the Rockefeller Foundation. So successful was the project that in 1959 Rockefeller joined forces with the Ford Foundation to establish the first international agricultural research centre, the International Rice Research Centre in the Philippines. In 1966 the two foundations sponsored the second centre, Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) in Mexico, with two more centres following the next year.

By the late 1960s Rockefeller and Ford were each contributing \$3 million annually to the centres, and it became clear they could support no further expansion. In 1971 CGIAR was created at the suggestion of the World Bank to coordinate finances of the centres, which now number 13 in all.

CGIAR, which has a full-time secretariat provided by the World Bank in Washington, remains a completely informal organization with no constitution or charter: its decisions are reached only by consensus among its members.

Funding has changed drastically over the past decade. The US Agency for International Development is now the largest contributor (\$44.5 million in 1983), followed by the World Bank (\$19 million). The International Development Bank contributed \$8 million. The Rockefeller and Ford Foundations have both decreased their contributions recently together accounting for less than \$2 million last year.

But the difficulty for CGIAR now seems to be in living up to the early successes of the first institutes: by the late 1970s Mexican wheat was grown on 29 million hectares of land worldwide, and improved rice strains on 25 million hectares.

Tim Beardsley

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 26 October	Change
14	6	Biogen (Switzerland)	8	7	-1
2	1	Bio-Logicals (Canada)	1 1/4	1 5/16	+1/16
14 3/8	5 1/8	Bio-Response (USA)	7 3/4	6	-1 3/4
14	9 1/4	Cetus (USA)	11	10 1/4	-3/4
10 3/8	4 1/4	Collaborative Research (USA)	5 5/8	4 1/2	-1 1/8
19 7/8	11 1/2	Damon (USA)	14 3/8	12 1/8	-2 1/2
26 1/4	11 3/4	Enzo-Biochem (USA)	14 1/2	18 1/4	+3 3/4
10 1/8	4	Flow General (USA)	5 1/4	4 7/8	-3/8
42 1/4	28 3/4	Genentech (USA)	30 1/4	9 7/8	-1 7/8
10 3/4	4 1/2	Genetic Systems (USA)	6 3/4	6 1/2	-1/4
17 1/4	6 3/4	Genex (USA)	9 3/4	7 1/8	-2 5/8
23	11	Hybritech (USA)	13 1/4	15 3/4	+2 1/2
16 1/4	6 1/4	Molecular Genetics (USA)	7 3/4	7	-3/4
15 1/2	8 1/4	Monoclonal Antibodies (USA)	10 3/4	10 1/2	-1/4
60 7/8	20 7/8	Novo Industri A/S (Denmark)	33 7/8	20 7/8	-12 1/2
22 3/4	14 1/2	Pharmacia (Sweden)	17 7/8	16 1/4	-1 5/8

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. *Nature's* weighted index of biotechnology stocks stood at 121 on 26 October, compared with 139 a month earlier. Data from E.F. Hutton, Inc.

Confusion about Chinese names

SIR — Richard J. Wang's note "Xiaoping first?" (308, 768; 1984) only scratched the surface of the issue of Chinese family names in romanization. In the five articles detailing results of the French-Chinese study of the North Himalaya-Tibet area (308, 17-36; 1984), only the second has authors' names correctly abbreviated in the table of contents. Admittedly, it is difficult for the uninitiated to know (or even make an educated guess) at which is the family name.

Confusion begins because the Chinese custom in Chinese is to put family name first, the opposite of the European custom. Added confusion comes because in romanization some Chinese westernize name order (family name last) while others do not. Further, some Chinese hyphenate the *given* name because it is two-character (confusing at first because Europeans sometimes take a hyphenated *family* name), while others (particularly in mainland China) fuse the romanization into one "word". Still others make three separate words for their name, so that the given name would be two words, always to be used together. This last practice is the most confusing since there is no cue to guess the family name; in the other methods, once realized, the hyphen, or the length of the word, allows the given, and thus the family, name to be identified. Even if one is familiar with the common Chinese family names, there are several distinct, widely-used, romanization systems (so Xu = Hsu = Shyu). Further, while pinyin is the sole romanization taught in mainland China, many Chinese elsewhere are not taught any particular system at all, so they sometimes show noticeable creativity. Knowing common last names in one system certainly helps, but not all the time.

The issue raised by all this confusion, particularly in the professional literature, is much more than that of slighting persons or cultures. It can and has already created a mess in indices. For example, to look up articles by Li Tindong in an index or abstracting service, does one look up L. Tindong, as given in that *Nature* table of contents and index. Or is it T. Li? I do not think it would happen, but if an editor insisted on consistency, it could also become T.D. Li, or T.-d. Li, or T.-D. Li. What is one to do? Even the thought of tediously searching each alternative is tiring, not to mention expensive if done through computer hook-up.

As an international journal, *Nature* should not be making these mistakes. Even after Mr Wang's letter I found an article by Zhang Yun (309, 547; 1984) listed in the table of contents and the mid-year index (310, 26 July 84) as Yun, Z. Poor Professor Zhang! Filed under first name. Ignorance could be claimed legitimately by my next-door neighbour, but by *Nature*? Clear, fair instructions to the authors, and clear, fair

editorial rules need to be established to handle these problems. Tell authors to circle family names, or, to indicate that last name, with or without hyphen, will be assumed familial unless otherwise indicated. Then give an example. Errors could be picked up, such as where R. Xu (in the first of the five articles mentioned) inverted his name apparently only because it is last in the list, even though it appears *Nature's* policy not to invert name order because of placement in a list of authorship. Consultation with librarians at an institute for Asian studies might help. After all, *Nature* does not abbreviate L.M. Van Valen as L.M.V. Valen (table of contents 5 January 1984).

I also suggest that indices should include the full romanized Chinese name. There are strong if not compelling reasons to support it. First is the lack of diversity in Chinese family names. Anyone familiar with Chinese names knows how widespread are a small number of family names such as Wang, Chen, Li, Zhou and so on. Indeed, in romanization the situation is worse than in Chinese because homophones (same sound and tone, different character) are so common. In addition, widely-used romanizations do not distinguish total differences, causing further redundancy. The only mitigating factor is

that use of different romanization schemes adds some variability. Chinese given names are quite varied, but all the points on romanization apply here as well. The habit of complete fusing of the given name further reduces variability when the given name is initialized. So the combination of initialling and the commonness of most family names must lead to immense redundancy.

Finally, Chinese names are short. Most are three characters — one familial, two for the given name — a few such as Zhang Yun are two, though a few others are four — for each name. Thus, one would be hard-pressed to find a romanized Chinese name over 15 letters. This is not true for names in many other languages, for example N.C. Vamvakopoulos (table of contents, 5 January 1984). *Nature* has recognized this since Chinese names are given in full at the top of all articles. The policy needs to be extended, perhaps not to the table of contents (so people know the family name!), but definitely to indexes.

With the increasingly large numbers of Chinese names coming into the western scientific literature, there are wide ramifications for information retrieval, not to mention human dignity.

JAMIE HOOK

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Student fees in Britain

SIR — When a nation is under financial strain, economies are necessary even in an important sector such as scientific research, which in the long run is one of the best investments. One could go further, admitting that financial restrictions, to some extent, may even be beneficial to research, in that they stimulate competition, force a better selection of people and projects and favour a more rational utilization of equipment and resources.

There is one measure, however, to which I would like to call your readers' attention, namely, the higher fees imposed on university students who are not resident in the United Kingdom. Apart from any question of reciprocity (I do not think that British students are requested to pay higher fees in any foreign university), I think that such a rule will ultimately damage British science. I know a case, by no means unique, of a British subject, not resident in the United Kingdom, who, having graduated *cum laude* abroad, wanted to study for a PhD in Britain. She would have been accepted by two leading university departments but at a cost of several thousand pounds. As a result, she accepted a research assistantship leading to a PhD, with an adequate salary, in a department of excellent reputation in another country in the European Community.

This is only one example of how foreign

students and graduates are actually discouraged from coming to Britain. Gifted students at least, especially at postgraduate level, should be attracted, even at the cost of some money. First of all, graduate students do productive work, which would be very cheap indeed if the nation has not borne the cost of their previous support and education. Secondly, when returning home these people will take away with them for life the memory of the country and of the institutions in which they were trained. This will result in the arrival of other young people and in the selling of equipment and may have even greater political implications. Last but not least, many of the best will stay. In this context, there is no need to mention what the United States has gained by recruiting foreign scholars and scientists, often in their youth.

I hope this will not be considered as an undue intrusion into the affairs of a country where I often was and am currently a guest. Indeed, for the gratitude I have for what I learnt and received in your country I felt a moral obligation to express a disinterested opinion on what seems to me a very serious matter.

FIorenzo STIRPE

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Civil aviation

SIR — Your leading article on 6 September, p.1 ("Poor show for aviation industry") complains that "civil air transport is not as accessible or cheap as it should be". Perhaps the judgement would be fairer had the article ended "as I would like it to be". The author has clearly not grasped the complexity of an airline operation. The technology necessary to take people around the world near or above the speed of sound costs money, and to do so safely requires one of the most intricate man-made systems most of us encounter. Add to this the requirement to ensure that flying is a positive experience for the customer and that there is a reasonable profit and to cope with political pressures from many governments.

As a pilot with a major international carrier I would hesitate to comment publicly on the financial wisdom of subcontracting aircraft component manufacture, an area which I recognize as only indirectly related to my area of competence.

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Indian science

SIR — I would like to add to the explanations which have been given in your columns of the poor quality of research in Indian universities and institutes. The problem at many Indian universities and institutes is a lack of understanding concerning the philosophy of higher education and a lack of sincerity on the part of the academic faculty and administrative staff. There is also a perversion of the professor-student relationship into what often resembles a master-servant relationship.

Many senior scientists from Indian universities and institutes work hard while in the West but when they return to India depend heavily on technicians and students in their laboratories. Another contributing factor is that scientists plan frequent long trips to the Western institutions. Their time in India is mostly spent on collecting money and making arrangements for their next trip. This situation deprives doctoral students of proper guidance from their professors. Often dissertations are presented to the university by the doctoral students in the absence of their major professor.

The concept of higher education in many Indian universities is misunderstood. Comprehensive solutions are required. Despite a huge cut in government support for scientific research in Europe and the United States during the past few years, the number of publications in a scientific journals is increasing. On the other hand, the Indian Government is spending a much greater percentage of the gross national product on science and technology than in

many other nations. Scientists and administrators in India cannot simply sit in their offices cursing the poverty of the Indian economy. In my opinion, the solution lies in increasing the commitment of all those involved in science.

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Scientists' oath?

SIR — Television dramas such as *Threads* and *The Day After* make us painfully aware of the great power that scientific and technical knowledge has for evil as well as good. A crucial question is the extent to which scientists, as individuals and as a profession, accept social and ethical responsibility for their research. I suggest that scientists, on graduation, take an oath or make an understanding that their work be for the benefit, rather than detriment, of mankind. Though such an oath would not legally prevent scientists from working on, for example, new nerve gases and new nuclear warhead systems, it might make a start to the establishment of a code of ethics for the scientific profession and bring this body into line with older institutions such as medicine and the church. Such a scientific Hippocratic oath might influence decisions made by individual scientists and a scientific code of ethics might answer an increasingly more popular groundswell of opinion that scientific "progress" *in toto* is not worth the associated risks to the survival of the human species.

JONATHAN HOWARD

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Jobs for biochemists

SIR — Richard Pearson's article "Is all science vocational?" (*Nature* 310, 260; 1984) will no doubt be widely read and quoted. As regards the employment of biochemistry graduates, however, the figures obtained by the Biochemical Society, which has been conducting an annual employment survey for some years, indicate a somewhat less gloomy situation. Thus, in 1983, 12.4 per cent of new graduates were still unplaced at the end of the year with another 6.3 per cent unknown as regards employment. At least 80 per cent are known to have found useful occupation within 6 months of graduating, such as further biochemical training for higher degrees (25.4 per cent), biochemical employment (25.5 per cent), other further studies (9.7 per cent) or non-biochemical employment (9 per cent). In addition, 11.7 per cent went abroad, including overseas students returning home and British students taking jobs. Given the present general difficulties as regards employment

of both graduates and non-graduates, these figures suggest that prospects for biochemistry graduates remain reasonably good and apparently considerably better than for biologists.

H.R.V. ARNSTEIN

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RICHARD PEARSON REPLIES:

Professor Arnstein's letter provides valuable information about biochemists and highlights the range of employment they have entered in the past. However when making comparisons *between* subjects for example with biologists, it is important to compare like with like and to use common databases as was done in my article. While the statistics are capable of different interpretations, cross-referencing one source to another can lead to inappropriate comparisons.

Animal antibiotics

SIR — In the article by Stephen Budiansky on the emotive subject both sides of the Atlantic, "Are antibiotics in animal feed a threat to human health?" (*Nature* 4 October, p.407), a few statements cannot pass unchallenged.

For instance, the statement* that "none of the bacteria residing in the gut of farm animals is resistant" is just not true¹. Rather than looking for evidence that animal pathogens constitute the source of R plasmids for human pathogens, as is suggested, evidence already points to the R plasmid-carrying indigenous flora in the animal gut as the main reservoir². This reservoir is being selected continuously by the use of antibiotics for whatever purpose. The present debate, therefore, hinges on whether or not subinhibitory, rather than prophylactic or therapeutic, levels of antibiotics play the major role.

Further, the suggestion that the energy cost to bacteria of carrying R plasmids places them at a disadvantage is not scientifically substantiated. It is now recognized that many plasmids are stable entities of the bacterial cell. Also, antibiotic resistance determinants are sometimes genetically linked on the same plasmid with virulence determinants³ and consequently antibiotics select for both types of determinants.

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1. Linton, A.H. *Vet. Rec.* 108, 328-331 (1981).

2. Linton, A.H. & Hinton, M.H. in *Antimicrobials and Agriculture* (ed. Woodbine, M.) 533-549 (Butterworths, London, 1984).

3. Timoney, J.F. & Linton, A.H. *J. appl. Bact.* 52, 417-424 (1982).

*This should have read "not all of the bacteria. . .", but was inadvertently changed in the editing. — Editor, *Nature*.

AIDS casts a longer shadow

The good news is of rapid progress in describing what seems to be a new virus — the bad news, proof of heterosexual transmission and of the mounting prevalence of antibodies.

WHILE no patient has yet been cured of the disease called AIDS (for acquired immune deficiency syndrome), it is proper that there should be full-hearted celebration at what has been done to understand the mechanism of this infection. That is one legitimate response to the appearance of the latest contribution from Dr Robert Gallo, at the National Cancer Institute, and his associates there and elsewhere, in France as well as in the United States (see page 166, this issue). The latest development is that Gallo has been able to clone DNA copies of the RNA genome of the virus, called HTLV-III (for human T-cell leukaemia virus), which was identified as the causative agent of AIDS only a few months ago. Presumably it is now a matter of weeks, not months, before the full nucleotide sequence of the 10,000 or so bases in the genome is known. Quite soon, it should also be known whether the vaccines against AIDS will be effective.

So quick has been the succession of events in this saga that it is easy to forget that the disease was first recognized only as recently as 1981. Inevitably, hindsight showed that the disease was not entirely novel — people had been dying from it for at least a couple of years. The chilling feature of AIDS was, and remains, the high mortality among those affected. In advanced societies, there is still no basis on which to deny that those frankly infected will in due course die, usually of some other infection or from Kaposi's sarcoma. That the disease, for the time being, is largely confined to a few groups of people (male homosexuals, those who inject themselves with narcotics and those dependent on blood transfusions, haemophiliacs especially) does not make the social problem less serious or even allow those not in one of the groups at risk to congratulate themselves that they will never come to harm. If, as is probable, HTLV-III is a virus to which the populations of advanced societies are still naive, it is understandable that it should first infect those to whom transmission is easiest. By the same logic, spread will eventually be more general. AIDS should be everybody's worry.

That is another reason for marvelling at the speed with which so much has been learned about the virus, and at the luck that AIDS did not strike ten years earlier, when it would have been much more difficult to disentangle what is going on. One of the crucial steps in characterizing the disease has been the recognition that an almost

invariable accompaniment of overt AIDS is an altered balance between the T lymphocytes called "helpers" and "suppressors". (There are fewer helpers.) These white blood cells are concerned with the regulation of the immune response in ways that are by no means clear. But ten years ago, even the distinction between helper and suppressor cells had not been established.

For the rest, the development of the present understanding of AIDS, and the description of HTLV-III, has followed the path that would have been expected of classical virology — but at an enormously accelerated pace. Gallo's group was able, in 1981, to hit the ground running because of the experience it had acquired in its investigation of the virus now known as HTLV-I, responsible for the disease called adult T-cell leukaemia, first recognized in south-west Japan but then among Caribbean and some African populations. That also upsets the balance between helper and suppressor cells, so that it is not surprising that Gallo and his associates can now also report that the genetic structures of the two viruses have much in common but are also distinct. If that investigation had not been fresh in their minds, it is hard to think that Gallo's group could have made such rapid progress.

But Gallo has not been the only investigator in the field. Professor Louis Montagnier's group at the Institute Pasteur in Paris was formally the first to announce that AIDS is caused by a distinctive virus, in May 1983. That virus, christened LAV, has now been shown to be indistinguishable from HTLV-III, and is also being produced by an infected cultured cell-line. There is even said to be some tension between the two groups. In August this year, Professor Montagnier wrote (see *Nature* 310, 446) in terms suggesting that he and his associates feared they would be overlooked in all the attention being paid to Gallo's fast-moving high-tech enterprise. That would be absurd as well as unjust, for in spite of what has been learned, so much remains to be learned about the biology of AIDS that everybody's energies will be needed before we are safe.

Mercifully, there is movement even towards that goal. Gallo and his associates have reported (*Science* 226, 447; 1984) the presence of HTLV-III in the saliva of apparently healthy male homosexuals and in that of people suffering from what is

called ARC, or AIDS-related complex (which may or may not be an early form of the disease). They have also recovered HTLV-III from the semen of two AIDS patients (*ibid.*, p.449). Guesses at the method of transmission by male homosexuals are thus confirmed; the bad news is that apparently healthy people can be carriers (and presumably transmitters).

It remains perplexing that so little can at present be made of the prevalence of antibodies against HTLV-III among apparently healthy people. Most AIDS patients carry antibodies against the virus, suggesting that their first response to infection was normal, but that their immune system was eventually overwhelmed. There seems to be abundant evidence (little of it published as yet) that the presence of antibodies is widespread among the groups at risk. Only a proportion of those infected by HTLV-III may succumb to AIDS, but, with the long lapse of time (perhaps four years) between infection and overt disease, it is not yet possible to tell how many of them will develop AIDS. Only prospective studies can serve.

Montagnier and the other members of a Franco-Belgian team have made an indirect and preliminary start on this part of the problem. Working in Zaire (once part of the Belgian Congo), they have traced the origin of the first AIDS cases presenting themselves at hospitals in Belgium in 1981. It now emerges (Brun-Vezinet *et al.*, *Science* 226, 453; 1984) that as much as five per cent of the population of Zaire may carry antibodies. By using sera kept in hospital refrigerators from earlier investigations, it has been possible to date one case of AIDS to 1977. The same team (Piot *et al.* *Lancet* ii, 65; 1984) has found unambiguous evidence of heterosexual transmission — and an annual incidence of AIDS as high as 20 per 100,000.

On the face of things, AIDS is nevertheless a new disease. Its nearly simultaneous appearance in Zaire, Haiti and the United States suggests that HTLV-III itself is a novel virus, presumably derived from something like HTLV-I. But how? And why does infection have a profound effect on only one class of T cells? Why should Kaposi's sarcoma, normally rare, be such a common sequel?

For the time being, these questions are wide open. But we are fortunate that Gallo and Montagnier were so quick off the mark, and that they and others now have the techniques they need. **John Maddox**

Fluid flow

Turbulence splits polymers

from A. Keller and J. A. Odell

EVEN minute quantities of long-chain molecules can dramatically influence the flow properties of liquids to which they are added, an effect that has many practical applications. However, limits are set on the usefulness and range of applications of polymer additives by the fact that the longest polymer chains are more effective but are also most likely to break during flow. Horn and Merrill¹ report on page 140 of this issue that such degradation of long flexible polymers subjected to turbulent flow conditions occurs by breakage at the midpoint of the molecules. Such apparently simple observations of degradation are also providing an insight into the molecular processes underlying many problems in fluid transport.

The countless examples of the use of polymers to modify fluid flow range from thixotropic ('non-drip') paints to engine oil viscosity enhancers. The energy required to pump liquids in turbulent flow can be reduced by up to 70 per cent ('drag-reduction') by the presence of a few parts per million of flexible long-chain molecules²; this has found practical applications in pipelines, heating systems and sewage disposal. Recently polymeric aircraft fuel additives have been developed to prevent fuel from 'misting', thereby dramatically reducing the risk of fire in the event of accident³.

The scientific approach to these effects has mostly been that of formal macroscopic hydrodynamics. Explanations on a molecular level have been proposed but are largely untested. A common hypothesis is that the effects are associated with the stretching out of flexible long-chain molecules in the course of flow. Since this process absorbs energy it could suppress droplet formation in misting, or defuse the nucleation of critical vortices during turbulent flow.

The earliest predecessors of Horn and Merrill's observation of midpoint scission of polymer molecules concern DNA⁴⁻⁶, which is not a highly flexible chain, but a rather rigid worm-like entity. Clearly a rigid rod-like molecule in any flow field will experience the greatest force at its centre, so that midpoint scission can be anticipated. But the more common flexible molecule chains are initially present as a tangle (random coil) and their scission would be expected to occur randomly along the chain. Only if the molecule is first straightened out can it be expected to behave as a rod and break at midpoint.

The systematic achievement of full stretching of chains through flow is by no means trivial, as conventional simple shear flows (such as laminar flow in a capillary or between rotating concentric cylinders)

merely distort the coil but do not straighten the chain. Instead, it is necessary to use special elongational flow systems in which there is a uniformly accelerating flow of very high velocity gradient (strain rate). Our own studies⁷ have shown that at a critical strain rate, which depends upon molecular length, the molecules undergo a sharp transition from the random coil to the fully extended state. At a higher and fixed strain rate, the increased force on the stretched-out molecules ruptures them at midpoint. The strain rate required to produce scission is proportional to the square of the length of the chain. This is predictable from the stresses arising in a stretched-out chain subjected to extensional flow. The deduced rupture strength of the chain is comparable to the strength of a covalent bond in the backbone.

This close correspondence between the fracture behaviour of coiled polymers under these model flow conditions and that of a straight molecule provides strong evidence that the coiled chains have been fully stretched out. Merrill and Horn, having corroborated these results and extended them to laminar flow through constrictions⁸, have now transferred the ideas and methodology to the less well

defined, but perhaps practically more important, condition of turbulent flow.

It has been hypothesized that in turbulent flows elongational flow fields prevail locally in vortices, producing local chain extension⁹. This cannot be observed directly on the microscale within the otherwise inhomogeneous turbulent flow, as could be done in the laminar model flow systems. Yet, as now reported, the unique effect of such flow and extension in the form of midpoint scission is observed⁸. By analogy to the experiments with model flow systems, Horn and Merrill infer that the chains must indeed have been stretched out by the turbulent flow field.

With the behaviour of macromolecules in turbulent flow now becoming accessible to experiment, we have reached a firm starting point for a molecular understanding of the intriguing and important flow-modifying effect of macromolecules and its consequences for fluid transport. □

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Molecular neurobiology

And now the sodium channel

from Charles F. Stevens

THE cloning and sequencing of the cDNA of the electric eel's sodium channel, described by M. Noda and seventeen colleagues on page 121 of this issue¹, reaffirms that S. Numa's laboratory at Kyoto University is the preeminent factory for cloning peptides and proteins of importance to neural function. More importantly, it is a key step in revealing the molecular basis of the electrical excitability of neural tissue and muscle.

What is the sodium channel and why is it important to clone and sequence its DNA? Channels — as the proteins responsible for brain electrical activity are called — fall into two nearly mutually exclusive classes: ligand-gated and voltage-gated. The former, when bound to by their ligands (specific neurotransmitter molecules) open to permit ion flux across the neuronal membrane. By contrast voltage-gated channels open and close in response to the voltage difference between the inside and outside of the neuron. The archetypal ligand-gated channel is the acetylcholine receptor (AChR). Numa's laboratory was the first to clone and sequence all four

subunits of AChR, and is now first to clone and sequence an example of a voltage-gated channel. In picking the sodium channel, they chose the membrane protein responsible for the nerve impulse.

Not only do AChR and sodium channels fall into different physiological classes, but they also belong to different structural classes. Whereas the AChR is a pentameric protein composed of structurally similar subunits, the picture of the sodium channel that emerges from the cDNA sequence of Noda *et al.* is of structurally similar domains within a single protein. The sodium channel of the electric eel is thought to consist of a single protein of >200,000 molecular mass. The cDNA sequence of this protein shows it to contain four domains, each of which is 250–300 amino acids in length and about 50 per cent homologous with the other three domains. Each domain has a similar overall structure with 6 identifiable subregions; two of these subregions are hydrophobic and presumably membrane-spanning, two are negatively charged, one has a large net positive charge, and one has no net charge.

The AChR is, cell biologically speaking, well behaved. To guide it through the endoplasmic reticulum during synthesis, it has an amino terminal signal sequence of the standard type for an integral membrane protein of eukaryotes. The sodium channel is more of a maverick in that it lacks an amino terminal signal sequence, as do a few other integral membrane proteins including ion pumps^{2,3}, the red cell chloride channel⁴, rhodopsin⁵ and the enzyme, 3-hydroxy-3-methyl-glutaryl coenzyme A reductase⁶.

Where do we go from here? The first and most important step — one which will not be long in coming — must be to demonstrate that the entity whose sequence Numa's laboratory has deduced can, in fact, function as a sodium channel. This proof of function will come from expression experiments of the sort described by Miledi and Parker⁷: mRNA from the cloned cDNA, obtained either by transcription or hybrid selection, will be injected into frog oocytes, which will make the protein and insert it into their surface membranes. If the protein produced is really a whole sodium channel, it will be detectable by electrophysiological techniques.

Another high-priority experiment will be to obtain cDNA clones of other sodium channels, especially the mammalian one, perhaps through their cross-reactivity with the eel cDNA. One interesting strategy will be to look for mRNAs that code for unusually long strings of glutamates — the eel sodium channel cDNA has nine in a row, starting with residue 942. (This approach may also be a route to other voltage-gated channels and a way to start defining family relationships.) The extension from eel to other species is important both for the comparative information that will result, and because the function of the eel channel is hard to study with modern methods, whereas mammalian channels are better understood and more easily studied.

Some aspects of the structural features of the channel deduced by the Numa group are unattractive. For example, it is known that the coupling between membrane field and channel-gating is achieved through a change in the protein's dipole moment associated with the transition between open and closed forms of the channel. This dipole moment change is unusually large (hundreds of Debyes for the sodium channel as compared with tens for the AChR) and must reflect some unusual structural feature of the protein that places movable charges within the membrane, where they will feel the electric field.

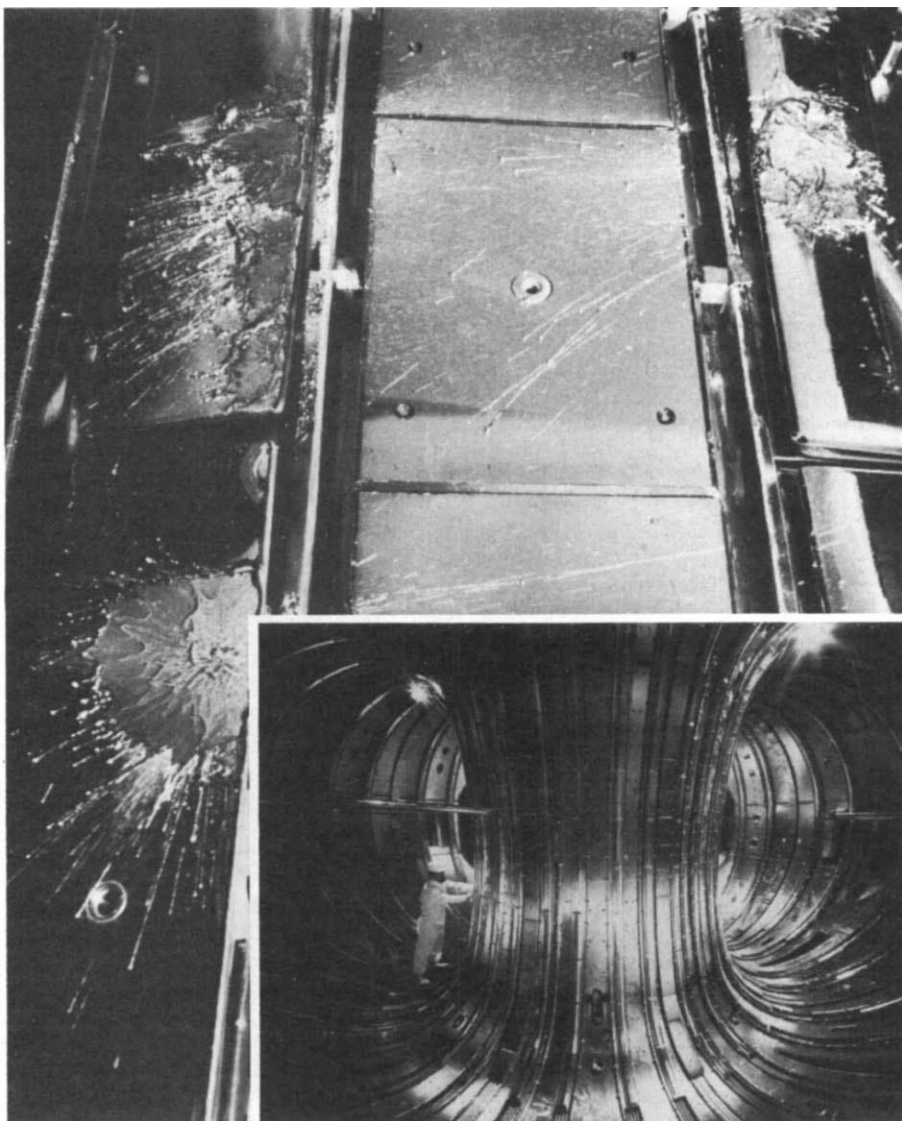
Unfortunately, no such feature is discernible in the structure suggested by Noda *et al.* As with the AChR channel, early efforts will be directed towards exploring alternative structures more in accord with physiological constraints.

Finally, we can look forward to structure/function studies of the channel, by means of site-directed mutagenesis of the cDNA. Numa's laboratory has a report in press in *Nature* using this approach for the AChR channel, and several other laboratories are carrying out similar experiments. The methods developed for the AChR will find quick application once clones for the sodium channel become more widely avail-

able. These are exciting times indeed for those with any interest in how the channels that control neural function operate. □

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Some 260 times since March, plasma pulses at the Joint European Torus (JET) in Culham have ended disruptively. The spectacular effect, shown above, is instantaneous melting of the Inconel alloy wall of the vacuum chamber by a beam of electrons stripped from the plasma during the disruption. The beam circles the chamber around half-a-million times in near-vertical magnetic fields, picking up energies of a few tens of MeV, and finally hits the inner wall to dump kilojoules of energy in a few milliseconds. The molten and evaporated metal can 'poison' carbon limiters used to limit the outer radius of the plasma ring in later pulses. The damage may contribute to JET's 'impurity problem', when chromium and nickel ions in the plasma radiate energy in interaction with plasma electrons, and limit the degree to which the plasma can be heated for a given rate of energy input. However, JET has shown a better ability to recover from disruptions — returning to normal performance within two pulses — than other tokamaks that have suffered from the same problem and have sometimes required a matter of days to recover.

Erratum

In Grant H. Thomson's article 'Details of Soviet cosmodrome from space shuttle photographs' (18 October, p.607), the words in italics in the following sentence were omitted: "Buildings and signs of construction activity in an area on the north side of the runway are thought to be preparations for the Soviet 'space plane'."

Active galaxies

Assembling the cloudy jigsaw

from Andrew Lawrence and Martin Ward

SOME galaxies have compact sources of radiation at their nuclei which display violent activity on a variety of timescales. It is difficult to identify a unique birthday for research on these active galactic nuclei (AGN), but there was no doubt that Professor Don Osterbrock of the Lick Observatory, and a distinguished AGN researcher, was 60 in July. At a workshop* held in celebration of that event, there was a feeling of substantial recent progress in three main areas: the understanding of the glowing gas surrounding AGN; the mapping of the overall energy budget of AGN at all emitting wavelengths; and the realization that activity in galactic nuclei is ubiquitous.

AGN are easily recognised by the bright optical emission lines in their spectra, arising from surrounding gas clouds heated by the central source of radiation. To understand the physical condition of the ionized gas, such as its temperature and density, much use is made of the relative intensities of the emission lines. D. Osterbrock reviewed the by now large data base on this topic, much of it gathered by himself and his graduate students at the Lick Observatory. This work has provided the information essential for the construction of models of line emitting regions. Indeed, a 'heavy industry' in AGN theory is that of modelling the shapes and strengths of the emission lines. Predictions from the standard model of a collection of identical optically thick clouds generally match the data well but several pieces of evidence imply the presence of a continuous range of densities and sizes in these clouds. In particular, B. Peterson (Ohio State University) argued from the variability of emission lines in AKN 120 that the clouds are uncomfortably close to the central radiation source and must evaporate, unless some of them are of a very high density. And W. Mathews (University of California, Santa Cruz) argued that symmetric Lyman alpha profiles further imply a substantial population of thin clouds.

Another outstanding problem is that of permitted ion emission, Fe II. This atomic species has an incredibly rich line spectrum in both the optical and the ultraviolet, but the spectrum is hard to model because, in certain parts, the multitude of Fe II lines can blend together to produce a false continuum (H. Netzer, Wise Observatory, Israel). In broad line radio galaxies, where the lines are even broader than usual, this may explain why Fe II was thought to be weak or absent. Since the lines are so broad they are 'washed out' and become part of

an apparently smooth continuum (B. Wills, University of Texas).

The profiles of these broad emission lines, produced by very fast cloud motions, must hold clues to the kinematics and geometry of the cloud regions. Much evidence points to radial motions but optically thick clouds, which would be bright on the side facing the central radiation source and dark on the back, should then lead to very different red and blue emission line wings. Because that is not usually observed, it further implies a mixture of thick and thin clouds. A. Filippenko (California Institute of Technology) and W. Ku (Columbia Astrophysics Laboratory) showed that lines from different atomic species often have very different profiles, again implying a physical stratification of the cloud regions. However, an interesting new idea regarding the line symmetry problem invoked the possibility of parabolic orbits, so that clouds are moving both out and in (J. Kwan, University of Massachusetts). And T. Kallman (Goddard Space Flight Center) suggested that in presence of a gaseous intercloud medium, scattering of ionizing photons could illuminate the back of clouds, making them bright on both sides. Finally, the radial motions need not be spherically symmetrical. M. de Robertis (Lick Observatory) presented a new model of a bi-conical expanding cloud region; viewed from different angles this could produce the various line profiles observed.

Energy budget

The second area of progress has been in understanding the continuum radiation from the central object itself. What is observed through a specific frequency window is often a mixture of components, such as synchrotron radiation, heated dust grain emission, atmospheres of hot young stars, and possibly thermal emission from a hot accretion disc surrounding a black hole. The sorting out of these different components, and their relative contributions to the total energy output of AGN, is now a major thrust of observational effort.

M. Malkan (Steward Observatory) argued for a 'thermal excess' in the blue over an underlying power law (the so-called 'blue bump') that may represent the radiation from an accretion disc. Others believe that this may be explained away by substantial Fe II and Balmer continuum emission from the surrounding clouds. M. Elvis (Smithsonian Center for Astrophysics) presented new soft X-ray data on optically selected quasars. The usually expected flattish X-ray slope was often not seen — rather, a variety of considerably steeper X-ray slopes were observed. Placed in the context of IR, optical and UV data, it

seems that the soft X rays in these cases may represent the high energy tail of the thermal excess postulated by Malkan. Furthermore a second 'little bump' is often visible on top of the 'big bump'. This presumably is the Fe II and Balmer continuum emission. This seems to resolve some of the controversy about the 'blue bump', in that we must be clear which 'bump' we are discussing.

J. Bechtold (Steward Observatory) and M. Malkan showed that the relative amounts of power-law and thermal components can be quite different in different AGN. Correlations within the properties of the power-law and thermal components, when considered separately, are generally good. M. Ward (Institute of Astronomy, Cambridge), using plots of the total energy output of AGN, emphasized that in many cases much of the energy must emerge at the peak of the thermal component in the extreme ultraviolet region (XUV). Unfortunately, this is currently an unobserved region — we see only the low energy UV head, and the high energy X-ray tail. To understand its true nature we need to observe the XUV body.

The third important area of progress is in recognizing that activity in the nuclei of galaxies is really widespread. As a result of sensitive spectroscopic surveys by W. Keel (Kitt Peak National Observatory) and T. Heckman (University of Maryland) it seems that 30 per cent of spiral galaxies have optical emission lines. This ionized gas cannot be produced by the radiation field of a population of normal semi-evolved stars. The required ionizing radiation could come from a small number of young, extremely hot massive stars, or be a dilute version of the power-law continuum radiation seen in quasars, or be shocked gas resulting from gas cloud collisions. Keel presented fresh evidence that these weakly active galaxies tend to have close companions somewhat more often than non-active galaxies — but the situation is confused. The presence of close companions seems to be a statistically significant factor, but is very far from being either a necessary or a sufficient condition for activity.

Much debate centred around the nature of the so-called Type II Seyferts. These are similar to the classical quasar-like AGN (most of which are classified as Type I Seyferts), but without the very broad emission lines or strong optical-UV-X-ray continuum. What remains are the strong narrow emission lines, and copious IR emission, almost certainly from heated dust grains. These are both features believed to come from the very large surrounding low density region. It is as if Type II Seyferts were Type I Seyferts with the energetic middle bits missing. D. Osterbrock and collaborators have long emphasized the existence of 'intermediate' types, with relatively weak broad lines and continuum, and evidence for a continuity of properties in several frequency regions was presented by A. Lawrence (Royal

*16-27 July 1984, Santa Cruz, California.

Greenwich Observatory) and R. Cohen (University of California, San Diego). However, A. Wilson (University of Maryland) argued that there exists a distinct difference in radio properties between Type I and Type II Seyferts. Some Type II Seyferts are really Type I Seyferts obscured by gas and dust; others may be 'switched off' Type I Seyferts — when the central radiation source switches off, the compact high density, high velocity cloud region will soon also disappear, but the very large low density regions may remain glowing for from hundreds to a few thousand years. Evidence for AGN changing their type on a timescale of decades was shown by Lawrence, Cohen and N. Bochkarev (University of California, Berkeley).

At least some of the classical Type II Seyferts may be quite different. J. Miller (Lick Observatory) reported that the Seyfert Type II NGC1068 has a highly polarised non-stellar continuum which extends through to the UV and soft X rays.

The emission lines are less polarised and those preferentially emitted from low density gas (the so-called 'forbidden lines') are polarised at a different angle from the permitted lines. This suggests that the central high density gas is present after all but for some reason is not moving at the great velocities characteristic of Type I Seyferts. G. Ferland (University of Kentucky) presented UV data for several Type II Seyferts which further showed that they do have a non-stellar continuum but that it is very weak.

Sitting through the workshop, one had the feeling of assembling a giant jigsaw puzzle. We have at least the majority of the pieces and have completed the edges. Now we must take a deep breath before attempting to make sense of the picture in the middle. □

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Neurobiology

Memory and molecular turnover

from Francis Crick

RECENT spectacular advances in molecular biology will, before long, begin to make a massive impact on certain key problems in neurobiology. A synapse is a complex piece of molecular apparatus, but there is now no technical reason why all its components — not merely the major ones — should not be isolated, sequenced and characterized. We can expect to have a detailed knowledge of the many ion channels, receptors and so forth, almost at will, for any type of neuron in any species. All this, together with techniques such as patch-clamping, should put the behaviour of synapses on a solid foundation and in the process tell us something about the molecular basis of memory. However, in spite of recent advances in understanding memory, in both *Aplysia* and long-term potentiation in the hippocampus, one aspect is often overlooked.

The time span of human memory (without obvious rehearsal) is often a matter of years, sometimes even tens of years. Yet it is believed that almost all the molecules in our bodies, with the exception of DNA, turn over in a matter of days, weeks or at the most a few months. How then is memory stored in the brain so that its trace is relatively immune to molecular turnover?

Several possible solutions of the problem suggest themselves. For example, memory might be coded in alterations to particular stretches of chromosomal DNA. Much current thinking assumes that memory is stored, at least in part, in the 'strength' of (many) individual synapses, so this would imply that in any neuron there is a special piece of DNA for each of its synapses, generated perhaps by similar means to

those used in cells of the immune system. This seems unlikely. A related alternative is that there is a special *local* piece of DNA (or perhaps RNA) for each relevant synapse. For example, it is conceivable that each spine apparatus has its own piece of nucleic acid which is modified when the system needs to alter the strength of that synapse. This idea also does not seem very likely but might be worth bearing in mind, since each mitochondrion has its own piece of DNA.

Another alternative is that each synapse has at least one macromolecule which is relatively immune from molecular turnover. Again this does not seem very probable but such a molecule could be looked for by appropriate pulse-chase experiments during development.

Since none of these alternatives seems especially attractive, one is more inclined to suggest models that are cooperative in nature. That is, the molecules in the synapse interact in such a way that they can be replaced with new material, one at a time, without altering the overall state of the structure. It is easily possible to conceive many such hypothetical models. It occurred to me to think of the simplest.

Consider, for example, a protein molecule that is an essential part of a synapse and can exist in two states: active and inactive. Suppose the molecule forms a dimer, probably with a two-fold rotation axis, that can be modified chemically by, for example, the attachment of a phosphate group. Let us assume that when both monomers are modified the protein is in the active state, symbolized by (+, +) and that when both are unmodified the dimer is in-

active, symbolized as (-, -). In addition, let us assume the existence of an enzyme that will add a phosphate group (or whatever the modifier is) to one monomer if the other monomer of the dimer is already modified but not otherwise. That is, it will turn a (+, -) into a (+, +) but will not touch a (-, -).

Assume, furthermore, that the mechanism for altering the synaptic strength can phosphorylate the dimer when more parts of the synapse are to be made active and can dephosphorylate it when activity is to be reduced. That is, the mechanism can turn a (+, +) into a (-, -), or vice versa.

Finally I assume that individual monomers of a single dimer can be replaced one at a time by the mechanisms responsible for molecular turnover and that these new monomers are initially unmodified.

This system will have the property that whether the dimer is in the active (+, +) state or in the inactive (-, -) state, the substitution of a single new unmodified monomer for either of its two components will leave it in the same state. That is, molecular turnover will turn a (+, +) into a (-, +) but the hypothetical enzyme will convert this back to (+, +). The (-, -) state will of course remain (-, -) during metabolic turnover. This mechanism is obviously modelled on known mechanisms for the maintenance methylation of DNA, as reviewed by Razin, A. and Friedman, J. in *Progress in Nucleic Acid Research and Molecular Biology*, Vol. 25 (ed. Cohn, W.E.) 33 (Academic Press, 1981).

It is possible to imagine more complicated models of this general type. The macromolecule could be a trimer or a tetramer; there might be more than one modifiable site per monomer; rather than phosphorylation, the modification might be dephosphorylation, or methylation, glycosylation and so forth.

There are a number of subsidiary conditions. The macromolecule should be anchored to a particular synapse and not be free, in its mature form, to migrate from one synapse to another. This might suggest it would probably be a membrane protein and perhaps form part of a larger aggregate of some form. The supply of newly synthesized monomers should be immune to the modification (or demodification) process. The old monomers, due to be discarded by molecular turnover, should not interfere with the above processes.

Since the proposed mechanism is not implausible it would be sensible to look carefully for modifications to synaptic proteins and for the enzymes which modify and demodify them, in case one of these enzymes should have the peculiar characteristics described above. If such an enzyme were found it might prove to be a pointer to the seat of long-term memory. □

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Atmospheric carbon dioxide

Predicting plant productivity and water resources

from T.M.L. Wigley, K.R. Briffa and P.D. Jones

PLANTS love carbon dioxide. At elevated atmospheric CO₂ concentrations photosynthesis (and hence productivity) is enhanced, plants transpire less and make more efficient use of soil moisture reserves. In assessing the agricultural and hydrological consequences of anthropogenic CO₂ increases, we must, therefore, consider both CO₂-induced changes in climate and the direct effects of increased CO₂ on plant physiology. The latter could be as important as the former, and may well be more easily predicted. Two recent papers have addressed different facets of this issue. In one, LaMarche *et al.*¹ suggest that recent increases in the growth of some high-altitude trees might be attributable to the direct effects of increased atmospheric CO₂. In the other, Idso and Brazel² examine the effect of a CO₂-induced increase in the efficiency of water use by plants on river basin evapotranspiration and river flow. Because Idso and Brazel assume that controlled-environment experimental results can be transposed to the real world, it is particularly important to demonstrate the validity of their assumption.

Before describing the work of LaMarche *et al.*, we can estimate the magnitude of increased tree growth that might be expected to occur under laboratory conditions due to a 25 per cent increase in CO₂ concentration, the change that has occurred since the mid-nineteenth century. To do this we use the well-known relationship between net primary productivity (NPP, roughly equivalent to tree ring width times mean density in this case) and CO₂ concentration (C): $NPP = NPP_0 [1 + \beta \ln(C/C_0)]$. β in this equation must be determined empirically and its value is still uncertain. Using $\beta = 0.3-0.7$ (see ref. 3) the implied change in NPP should be 7-16 per cent. This is a sufficiently small change that it would be exceedingly difficult to detect, given the natural variability of plant growth.

In fact, β may be even less. The values quoted are based largely on small-scale experiments which, as noted above, may not be applicable to the real world. A more relevant value can be estimated from carbon-cycle modelling studies but modelling uncertainties are such that a β value as small as zero is not ruled out. Since carbon-cycle models are our main tool for predicting future CO₂ levels, and since these predictions depend on the chosen value of β , it is clearly of importance to detect enhanced photosynthesis in the unmanaged biosphere in order to demonstrate that β is significantly greater than

zero.

In their field study, LaMarche *et al.* examined variations in bristlecone pine ring widths at two high-altitude sites in the White Mountains of eastern California, approximately 370 km east of San Francisco, and in limber pine ring widths at a site high up on Mount Jefferson, Nevada, about 180 km northeast of the other sites. At all three sites LaMarche *et al.* found an increase in tree growth since the early nineteenth century and particularly over the last 20 years. For the two Californian sites, where the tree ring data extend back many centuries⁴, the changes are unprecedented. Furthermore, the increases in ring width shown by LaMarche *et al.* exceed 70 per cent over the past 150 years, much greater than any anticipated changes (unless there has been a correspondingly large reduction in wood density). This clearly points towards some unusual cause, and CO₂ is implicated because it too has shown an unprecedented increase over the last century. However, if CO₂ were the cause we could expect the effects to be widespread and relatively small. Other bristlecone pine and limber pine chronologies exist near to the sites studied⁴, but not all of these show the same upward growth trends. Further research is required to explain this site specificity and the apparently large amplification of the CO₂ effect. Could, for example, an amplified effect occur specifically at locations near the tree line if there were a CO₂-induced reduction in the threshold temperature for photosynthesis?

Alternatively, are these changes due to climatic change? LaMarche *et al.* consider only temperature and precipitation changes (neither show any recent trends), and their analysis is inconclusive because the climate-tree growth relationship is not well-defined. To complicate the issue further, climate may be indirectly involved; for example, since 1960 the regional westerly wind component has increased in a way that, at least superficially, matches the tree growth trends. Could changes in nutrient input due to pollutant transport from the industrial and agricultural areas around San Francisco be the link? While intriguing, the data of LaMarche *et al.* are not yet enough to support any claim that the growth changes are related to CO₂ increases, and the authors are justifiably cautious in their conclusions.

Idso and Brazel are not concerned with small past changes, but with the much larger increases in CO₂ that might occur in the future. They consider the case where the CO₂ level is double the pre-industrial

level — a value expected to occur around the mid-twenty-first century — and examine the effects of CO₂-induced changes in water use by plants in line with the NPP relationship given above.

Higher CO₂ levels reduce evapotranspiration by causing the stomata to close. When evapotranspiration is reduced, more precipitation will be available for runoff, so reducing the normal depletion of soil moisture reserves during the growing season. Thus, whereas the potential effects of any reduction in precipitation caused by increased CO₂ concentration could be offset via the direct effects of CO₂ on evapotranspiration, the effects of an increase in precipitation might be enhanced. To study the relative effects of these two processes requires both a knowledge of the future change in climate and a detailed model of the vegetation. Idso and Brazel use a simple water balance calculation, coupled with the climate scenarios assumed in an earlier study⁵, to assess the sensitivity of runoff to both effects. Their results indicate that direct CO₂ effects could more than offset influence of even such large changes in climate as a 10 per cent drop in precipitation coupled with a 2°C rise in temperature. In a much more complex analysis, Aston⁶ has reached a similar conclusion.

The changes in agricultural and hydrological resources predicted by these studies will have to be considered carefully in future planning. Both, however, are exploratory, and depend on a number of simplifying assumptions. Idso and Brazel, for example, take no account of the seasonal distribution of precipitation and evapotranspiration. If most of the runoff were associated with winter precipitation (for example, through snow melt) then a reduction in drainage basin evaporative losses during the growing season could have only a minor effect on annual runoff. Even during the growing season, the reduction in evapotranspiration may be offset by an increase in leaf (transpiring surface) area, a factor considered in neither study. Calculations by Goudriaan *et al.*⁷ using a very detailed plant physiological model, show that increased leaf area could largely compensate for reduced transpiration rate per unit leaf area. However, as Aston points out, this would not operate in tropical regions where C-4 plants often dominate.

These are all exceedingly complex issues. Future changes in atmospheric CO₂ level

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2. Idso, S.B. & Brazel, A.J. *Nature* **312**, 51 (1984).

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are expected to far exceed any changes that have occurred to date. Even in the absence of marked changes in climate, atmospheric CO₂ may have dramatic effects on agriculture and water resources. To support rational planning for this eventuality, we require more studies like those discussed here to increase our quantitative knowledge of the influences of CO₂. But

much more groundwork is required before we can make predictions that have practical value. We may have only a decade or so in which to make the step from speculation to application. □

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Palaeoecology

Hampstead Heath clue to historical decline of elms

from Peter D. Moore

LIKE human history, palaeoecology can be a frustrating occupation. How can one hope to construct a satisfactory, scientific explanation for an event which appears to have taken place on only one occasion in the fossil record? The whole basis of uniformitarianism begins to totter at this point. On the other hand, such events can fuel endless speculation, the pleasures of which tend to offset the frustrations. One of these has become a recurrent theme in the Quaternary palaeoecology of Europe: the decline in elm pollen which occurred quite abruptly across much of northern Europe, some five thousand years ago. It was this theme which reared its enigmatic canopy over a recent conference of botanists and archaeologists at Oxford*.

In the past few years, the debate has lost something of its vigour simply because no new evidence has come to light. It has been established that the collapse of the elm pollen in the stratigraphic record is widespread, relatively synchronous (within a 300–400 year period over a very large area, as indicated by dates which themselves, however, have broad inherent error margins). There are some isolated locations where the elm decline is less marked, but even in the extreme north of Europe, far beyond the limits of elm, it is often possible to detect a drop in the elm pollen that had been transported over considerable distances.

One of the major controversies has centred around the coincidence of the first clear indications of agricultural activity by Neolithic peoples with the elm decline, which has led to interesting speculations about primitive forestry practice and contemporaneous human population levels. Models advanced to explain the elm decline have evolved over the decades in a punctuated manner, each quantum leap being dependent on the acquisition of some new item of circumstantial evidence which permits further elaboration, or even a complete restructuring of the theory. Climatic change gave way to soil deterioration, which was overthrown by the radical

proposition that man stripped the elm trees of their branches in order to feed stockaded domestic herbivores.

After a period of equilibrium, if not stagnation, the topic seems set to make another leap, and the evidence is coming from a number of directions. In the first place, it has become apparent that agricultural activity in Britain was widespread prior to the elm decline. This fact was demonstrated at the Conference by the data of J. Innes and I.G. Simmons (University of Durham). Their work on the North York Moors clearly shows that there were episodes of forest clearance during what are presumed to be Mesolithic times. Evidently the advent of agricultural ideas and practices in northern Europe was not a sudden, revolutionary event. In a recent analysis of the first occurrence of cereal-type pollen grains, Edwards and Hiron (*J. archaeol. Sci.* **11**, 71; 1984) showed a wide geographical scatter of records in Britain prior to the elm decline. Whether these derive from Mesolithic or Neolithic cultural activity is impossible to say and may in any case be a rather meaningless question. What is important is that the earliest agriculture was underway before the elm decline, which was therefore not the outcome of any large-scale invasion of innovative, agricultural techniques.

A similar conclusion has been drawn by J. Turner (University of Durham) who displayed pollen diagrams based on contiguous 1 mm microtome slices of a frozen peat core from the North Pennines. These showed a progressive decline in elm, evidently related to the succession of clearance events which had been taking place in the region over a period of several hundred, possibly a thousand years. Since the elm decline is also accompanied by falling levels of lime (*Tilia*) and ash (*Fraxinus*), Turner is inclined to the idea that the cumulative effect of centuries of Mesolithic wear and tear on the vegetation has resulted in the depletion of soil nutrients, leading to a decline in all three species. She has also proposed, on the basis of the pore numbers in the fossil elm pollen, that the elm in question in the area was not the wych elm, *Ulmus glabra*, as

expected, but was more probably *U. minor* (see Stockmarr, *Danm. Geol. Unders.* IV R 4, No. 11; 1970). Since this taxon is climatically more sensitive than the wych elm, the suggestion could cause the entire debate to return to its starting point of some forty years ago.

It was at this point, however, that fuel was added to the fire from an unexpected quarter. M. Girling (English Heritage) announced the discovery of wing cases from an interesting beetle in sediments just 10 cm below the elm decline at a site on Hampstead Heath, London. The beetle she identifies as *Scolytus scolytus*, the carrier of the fungus, *Ceratocystis ulmi*, that is the cause of Dutch elm disease.

The proposal that the decline of elm 5,000 years ago was the product of a disease is not in itself new (see W.A. Watts, *Proc. Linn. Soc. Lond.* **172**, 33; 1960). It has become increasingly attractive, even compulsive, as we have observed the recent effects of Dutch elm disease on elm populations. But healthy speculation feeds upon circumstantial evidence and here, at last, we seem to have it. If the beetle vector was here 5,000 years ago, perhaps the disease was too. Was the sudden onset of the disease the result of early forestry? And how do we explain the concurrent decline in some other trees, such as lime? The answers are still not clear, and can never be entirely testable, but the new evidence will provide the necessary momentum for renewed vigour in an old debate. □

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100 years ago

NATURAL SCIENCE IN SCHOOLS

HOWEVER fully it may be admitted by the few that it is important, nay essential, that all members of the community, whatever their station or occupation should during their school career receive some instruction in the elements of natural science, the general public have not as yet had brought home to them with sufficient clearness that, just as a knowledge of foreign languages is essential to all who are brought into intercourse with foreigners, so in like manner is a correct knowledge of the elements of natural science of direct practical value to all in their daily intercourse with Nature, apart from the pleasure which such knowledge affords. In fact, from a purely utilitarian standpoint, the advantages to be derived from even most elementary acquaintance with what may be termed the science of daily life are so manifold that, if once understood by the public, the claims of science to a place in the ordinary school course must meet with universal recognition. To quote Huxley: "Knowledge of Nature is the guide of practical conduct. Any one who tries to live without attention to the laws of Nature will live but a very short time."

*Joint meeting of the Botanical Society of the British Isles and the Association for Environmental Archaeology, Oxford, 21–23 September, 1984.

Psychiatry

Complexities of cause and effect in anorexia nervosa

from Nicholas Mrosovsky

EXTREMES in body weight such as gross obesity or the emaciation of anorexia nervosa are accompanied by numerous physiological, behavioural and psychological changes. These disturbances are so interwoven that it is difficult to disentangle the primary factors. Classic studies by Sims and Horton¹ have demonstrated that, simply by overeating, volunteers of normal weight can obtain obese-type endocrinological profiles; when the period of overeating ends, body weight returns to normal and the endocrinological symptoms subside. Such findings suggest that, at least in some types of obesity, these particular symptoms are a consequence of being fat rather than instrumental in initiating overweight. This approach has now been applied to the problem of anorexia nervosa by Fichter and Pirke and their co-workers at the University of Munich and the Max-Planck-Institut für Psychiatrie².

The well-known Minnesota studies of Keys *et al.*³ have already shown that starvation induces a number of features that are common in anorexia nervosa; obsession with food and rituals surrounding meals are obvious examples. However, these observations were made on young men, while Fichter and Pirke selected subjects and measures with anorexia nervosa specifically in mind. Young women in their early twenties and close to 'ideal' weight volunteered to go without food, other than certain minerals and vitamins, for three weeks. A number of changes, reminiscent of anorexia nervosa, occurred during the fast, including elevated blood cortisol, abnormal dexamethasone suppression tests, blunted response to thyrotropin releasing hormone, lower levels of luteinizing hormone (LH) and regression of the 24 h LH secretory profile to the infantile pattern. The LH changes were not consistent. However, the average weight loss of 8 Kg was small compared with percentage reductions from ideal body weight commonly observed in anorexia nervosa. In general, apart from some less clear data with growth hormone, "the results support the hypothesis that the endocrine disturbances reported in anorexia nervosa are not primary but rather the consequences of reduced food intake or loss of body weight"².

While the results of the Fichter and Pirke study are consistent with the view that many physiological symptoms of anorexia nervosa are secondary to weight loss, there remain counter-arguments. The relationships of endocrine function to body weight in anorexics are by no means simple. For instance, weight recovery does not always

lead to normalization of LH levels⁵ and changes in the metabolism of brain nor-epinephrine sometimes last for months after nutritional rehabilitation⁵.

An abiding question about such counter-arguments is what do we mean by 'recovery'? Usually this is defined in terms of attaining a weight near to the ideal level for the age and height of the patient. This is far from satisfactory since it is not uncommon for anorexics to have been above average weight prior to the disease. Persistence of endocrinological abnormality in an individual might reflect a failure to recover to an appropriate weight for that individual. What is needed are indices of recovery other than average population weights from the Metropolitan Life Insurance tables. Even when the patient's own pre-morbid weight is used, there is the problem of defining the onset of the disease; moreover, anorexics often have unstable weights. Further studies of the effects of fasting and refeeding on people of stable weight, who do not normally diet, should provide better indices of deviations from an individual's own physiologically-regulated weight level. Without these, the relationship of endocrine function to body weight in anorexic patients may remain obscure.

Nuclear physics

From little bang to Big Bang

from Neil Rowley

AT sufficiently high incident energies, the system formed in a head-on collision of two heavy nuclei will 'explode', showering fragments in all directions. A new generation of particle detectors, which almost completely surround the target in such a collision, makes it possible to detect enough members of the shower to reconstruct some details of the initial event. Two experiments of this kind have recently been completed on the Bevalac at the Lawrence Berkeley Laboratory in California; the results of both hint at the possibility of some exciting new physics.

The first experiment, by H.A. Gustafsson *et al.* (*Phys. Rev. Lett.* **52**, 1590; 1984) employed the 'Plastic Ball' spectrometer to investigate the possibility of observing collective flow of nuclear matter in Ca + Ca and Nb + Nb collisions at 400 MeV per nucleon. The observation of such collective effects would give hope that some very exotic phases of nuclear matter could be created by relativistic

The idea of a strong biological component in anorexia nervosa has been entertained ever since the disorder was first described. Prospective longitudinal studies, with measurement of many physiological variable are costly and difficult. Given this vacuum, the experimental approach taken by the Munich group provides some of the most important information we have on the topic and may be destined to take its place alongside other classic studies on weight manipulation in normal subjects. Of course, these studies do not preclude predisposing factors or the possibility that a primary abnormality in metabolic regulation may result in weight changes which in turn produce secondary changes¹. Therefore the question of whether there are primary physiological disturbances in anorexia nervosa has not yet been resolved. It would be a fair summary of present knowledge to say that not one of the somatic symptoms has yet been proven to be primary in the disorder, and that a large number of these symptoms are not specific to anorexia nervosa, but can be produced by weight loss alone. □

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heavy-ion collisions.

In order to explore an equation of state and look for possible phase transitions, a large sample of matter needs to be taken and subjected to controlled variations of temperature and pressure. This is not so easy for nuclear matter since we only possess relatively small samples — the stable nuclei — and we can only vary the ambient conditions by forming hot compressed systems in short-lived nuclear reactions. Here the finite size of the nucleus is an apparent problem since nucleons with a high kinetic energy might rapidly escape from the compound system and thermal equilibrium may never be established.

At low excitation energies, the mean-free-path of a neutron or proton in a heavy nucleus is indeed large (at least when compared with the nuclear radius), though each nucleon is confined by the average field of all the others. Thus one might expect that highly excited nucleons would escape without scattering. The reason for

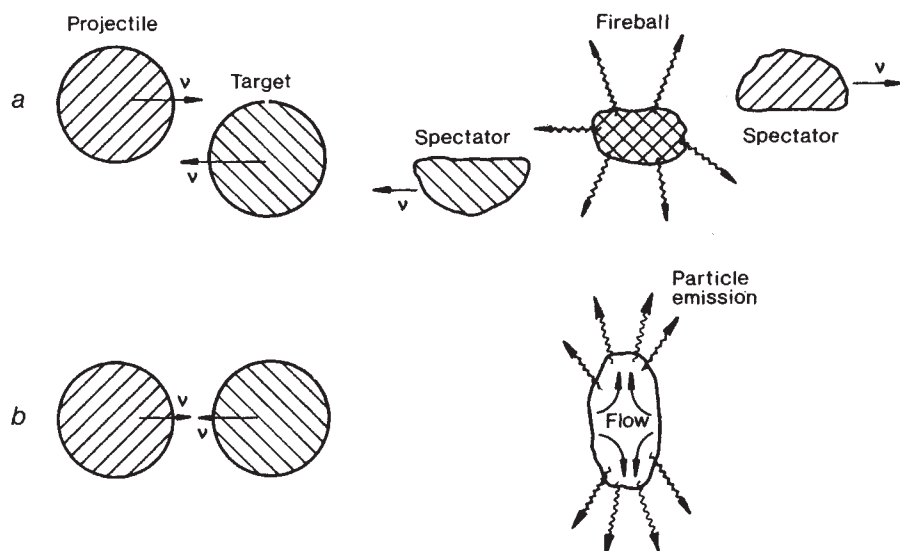


Fig. 1 High-energy collision of identical nuclei in the centre-of-mass frame. *a*, Non-central collisions. The sections of target and projectile which did not overlap remain as relatively 'cold spectators' moving with their original velocities. The overlapping sections form a 'fireball' whose centre-of-mass is at rest. There may, however, be collective flow in the fireball which decays rapidly by thermal particle emission. The final momentum distribution has the collective momenta superimposed on top of a statistical distribution from the decay. *b*, Head-on collision. All the mass is compressed into the fireball. In the hydrodynamical model this mass is squeezed out in a collective flow at right angles to the original beam direction. The system still decays by statistical emission but with an additional perpendicular component to the momentum. The flow may be very different if the target and projectile have significantly different masses (see text).

the large mean-free-path is, however, rather special. Nucleon-nucleon interactions are inhibited by the Pauli principle which prevents a neutron (proton) from scattering into a state already occupied by another neutron (proton). For very fast collisions many high-energy unoccupied states become accessible, the nucleon-nucleon scattering regains its full vitality and the nucleon mean-free-path becomes very small. At 400 MeV per nucleon the Pauli blocking may essentially be ignored.

It has already been established that in high-energy nucleus-nucleus collisions the emitted charged particles have an energy distribution which is consistent with a system that has fleetingly achieved thermal equilibrium. The observation of equilibrated particle spectra unfortunately tells us nothing about the internal pressure of the system. At the limit of small mean-free-path, however, a system should behave hydro-dynamically and it should be possible to set up local pressure gradients and generate collective flows. The problem is that long before reaching any detector, the hot 'fireball' formed from the overlapping regions of the target and projectile (see Fig. 1) will have 'radiated' away to nothing. The only way of knowing if such hydrodynamical effects exist is to detect as many as possible of the radiated particles, measure their momenta and reconstruct the flows existing before the fireball disintegrated. This was the aim of the experiment Gustafsson *et al.*

A further difficulty in interpreting the data from such experiments is that hydrodynamics predicts collective flows which depend on whether the collision is head-on

(see Fig. 1*b*) or more peripheral (Fig. 1*a*). The enormous power of a detector like the Plastic Ball lies in the fact that it is actually a large number (815) of electronically coupled detectors covering a solid angle of almost 4π , that is almost completely surrounding the target. By detecting many low-mass charged particles in coincidence with one another, it is possible to have a high degree of confidence that they originated from a single event and to relate qualitatively the particle multiplicity to the size of the fireball.

For identical nuclei the most dramatic event is the head-on collision — giving rise to high multiplicity. Here the hydrodynamical model predicts that the matter is squeezed out at right angles to the original beam velocity (see Fig. 1*b*). This event is referred to as 'sidesplash' and the direction of flow relative to the beam axis is specified by the flow angle. This should be 90° for a head-on collision. For the Ca + Ca experiment of Gustafsson *et al.* the results are disappointing. For all but the highest multiplicities the particle cross section is peaked at 0° , that is along the axis. This is consistent with a purely statistical decay and gives no evidence for collective flow. Only for the highest multiplicity events is a small deflection, of around 10° , observed.

For the more massive Nb + Nb experiment the results are much more encouraging. A finite flow angle is observed and it increases steadily with increasing multiplicity up to a maximum value of around 30° . This is far short of the expected 90° for a head-on collision but the occurrence of such a direct event is rather infrequent and the flow angle drops rapidly

to zero with decreasing projectile-target overlap. The theoretical analysis of Buchwald *et al.* (*Phys. Rev. Lett.* **52**, 1594; 1984) shows that when these effects and the detector efficiencies are taken into account a maximum average flow angle of about 30° means that collective hydrodynamical flow has been established.

More recently the mass-asymmetric system Ar + Pb has also been studied at the Bevalac in collisions at energies around 800 MeV per nucleon (Renfordt, R.E. *et al. Phys. Rev. Lett.* **53**, 763; 1984). Here an interesting twist to the flow-angle behaviour has been observed: for very central collisions (highest multiplicities) the fragment distribution is almost isotropic and there is no discernible flow angle.

Surprisingly, this result is again consistent with the hydrodynamical model which shows that the behaviour is due to the different 'geometry' of the system. In a central collision the Ar nucleus is totally engulfed by the larger Pb target in which it is brought to an abrupt halt. Since the kinetic energy is entirely deposited in the interior of the target this results in an almost isotropic expansion of the composite system. Statistical or 'cascade' models (see Cugnon, J. *et al. Phys. Lett.* **109B**, 167; 1982) do not produce such a large stopping power since they cannot describe the vital compressional modes. In these models some remnant of the initial beam velocity survives giving a forward peaking of the fragment distribution — a prediction inconsistent with the results of the second experiment and the Nb + Nb data.

For lower multiplicity events, however, the asymmetric system does exhibit a finite flow angle. The maximum value appears to occur for collisions where the Ar projectile is just eclipsed by the larger Pb target. This configuration now resembles the head-on collision for the mass-symmetric system, since the non-overlapping part of the target may be regarded as a spectator. The result is again elegantly reproduced in the hydrodynamical picture.

It does therefore seem possible to compress nuclear matter in such experiments. If compressions to around three times normal density can be achieved, then theory predicts the existence of a nuclear phase containing a Bose-condensate of pions. An even more alluring goal (though higher energy beams would be required) is the creation of a fireball in the phase of a quark-gluon plasma. Such experiments are our only possible means of reproducing in the laboratory a state of matter that only existed at the very beginning of the Universe. The creation of such exotic phases may still be far away but the new Bevalac results are an important step in the right direction. □

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Molecular evolution

On the origin of the *Alu* family of repeated sequences

from Andrew Leigh Brown

7SL RNA is an example of a eukaryotic RNA species that was identified long before a cellular role could be ascribed to it. Only recently was it established¹ that it forms an essential part of the signal recognition particle — the cellular apparatus that aids the transport of secreted proteins across the endoplasmic reticulum. Subsequently nucleotide sequence analysis revealed that parts of the 7SL RNA sequence seem to derive from the *Alu* element², which is repeated about 300,000 times in the human genome³. While *Alu* is usually found as a head-to-tail dimer of a sequence about 150 base pairs long, in the 7SL sequence a single *Alu* monomer seems to have suffered an insertion of 155 base pairs. This was not in itself a dramatic discovery because *Alu* elements are dispersed so widely throughout the human genome that they could easily be a target for random DNA insertion. However, the latest developments concerning *Alu* and 7SL RNA^{4,5}, some of which are published on page 171 of this issue, have taken a quite unexpected turn.

Ullu and Tschudi⁴ have sequenced 7SL RNA (as a DNA clone) from *Xenopus* and *Drosophila*. Both are clearly related to the human counterpart throughout their length — that is the sequence homology extends to the *Alu*-like part of the human molecule. The presence of an *Alu*-like sequence in *Drosophila*, also found by Gundelfinger *et al.*⁵, means that *Alu* has had a much longer evolutionary history than was previously thought. More interesting yet, in *Drosophila* it seems only to exist as part of the 7SL DNA, of which there are two copies. On this basis, Ullu and Tschudi suggest that *Alu* sequences represent defective 7SL RNA molecules that have been reverse-transcribed into DNA and inserted into the genome. An analogous origin has been suggested for alpha-globin pseudogenes in the mouse⁶, and the multiple pseudogenes for small nuclear RNAs in man⁷. Pseudogenes are generally thought not to play an important role in the cell. Perhaps those who have argued that *Alu*, by its very abundance, must have an important function will recognize that this argument has now lost some of its weight.

Although it seems likely that the 7SL sequence with its single *Alu* evolved in the absence of repeated *Alu* elements, an alternative interpretation of Ullu and Tschudi's data presents itself, at first sight. In the 7SL-specific region of the sequences the match between the human and *Drosophila* sequences is 67 per cent, and the correct alignment is obvious. In the *Alu*-related re-

gions the degree of match is less and the two groups opt for rather different alignments, especially at the 3' end. If, in fact, these regions of the sequence are completely unrelated, then the earlier hypotheses could still be tenable. However, using the method of Fitch and Smith⁸, I find that the alignment of the *Drosophila* sequence with the 3' *Alu*-related part of the human 7SL sequence suggested by Ullu and Tschudi has a probability of less than 0.01 of occurring by chance. Thus the flanking regions of the *Drosophila* 7SL RNA are homologous to *Alu* in man and the *Alu* sequence first existed as a part of this molecule.

As expected, for *Xenopus laevis* the overall match with the human sequence is much higher⁵ than either is with *Drosophila*, although it drops off in 3' *Alu*-related region. Although their previous results indicate only weak homology to *Alu* in *Xenopus* DNA⁹, Ullu and Tschudi describe the *Xenopus* genome as being 'rich' in sequences related to the *Alu*-like parts of the *Xenopus* 7SL molecule. So, in *Xenopus* as well as man, there is a close relationship between the 7SL RNA and highly repetitive elements, although the *Xenopus* and human sequences have diverged during evolution. How did this relationship arise? The fact that 7SL has a longer evolutionary history, being found in the absence of any *Alu*-related repeats in *Drosophila*, strongly suggests it was the antecedent. Moreover, the 7SL RNA is arranged in the signal recognition particle in such a way that three RNA fragments similar to the 5' *Alu*-related, 7SL-specific and 3' *Alu*-related sequence domains are generated by limited enzymatic digestion of the intact particle¹⁰. Put that together with the circumstance in which the 7SL RNA was first discovered — in a retroviral particle¹¹ and therefore close to a source of reverse transcription activity — and the generation of DNA copies of *Alu* is easy to imagine.

Amplification

Once *Alu* had appeared, what happened next? It has been known for some time that *Alu* is a remarkably stable component of the genome. Having arisen at a specific location, it does not seem to disappear quickly. For example, in the gamma-globin region, two *Alu* elements are present in exactly the same place in both man and chimpanzee¹². This is in striking contrast to the vagility shown by the middle repetitive DNA elements in *Drosophila*, and until now there has been no real explanation for the difference. However, if *Alu* originated

as a 7SL pseudogene it may not possess or respond to any mechanism for excision or transposition. Thus, unless it kills the chromosome in which it inserts, it might just have to stay there. If it retains polymerase III promoter activity (not all copies do retain it), it could even generate more transcripts, each of which might be another possible template for reverse transcription and reinsertion. One would therefore expect copies to accumulate in the genome.

Two questions are left. First, did the events which gave rise to the *Alu*-related repetitive elements occur once in each of the *Xenopus* and human lineages independently? If so, it might explain the sequence divergence between repeats of the two species; within the species the repeat sequence very closely resembles the 7SL sequence. However, we should remember that some concerted evolution has probably taken place. It is possible to envisage repeats already existing in the primitive amphibian DNA and gradually, after the two lineages separated, changing *en bloc*. Any subsequent generation of pseudogenes would be of the species-specific 7SL sequence; a process of concerted evolution which kept the 7SL and *Alu*-related repeats reasonably similar within each species is not unlikely.

The second question is, why has none of this occurred in *Drosophila*? Here there are no easy answers. The situation is similar to the case of the small nuclear RNAs, where many pseudogenes are found in man⁷, but not in *Drosophila*¹³. Processed pseudogenes of protein coding regions also seem to be absent in the fly. There is certainly no absence of reverse transcriptase activity which might explain this — generation of reverse transcripts may be a major mechanism for replication of *copia*¹⁴. Nevertheless, reverse transcripts do not accumulate in the genome of *Drosophila* in the way they have in vertebrates. When we know why this is so, we shall be well on the way to understanding why the genomes of insects are so very much smaller than those of vertebrates despite containing much the same amount of information. □

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Towards a model for the arms race

SIR — Saperstein's use of a discrete recursion model to describe the arms race¹ is intriguing. He uses equations derived from the study of chaotic behaviour, as manifested in fluid dynamics. We have two theoretical objections, but in addition, his model's predictions do not agree well with the available data.

The model states:

$$x_{n+1} = 4ay_n(1 - y_n) \quad (1)$$

and

$$y_{n+1} = 4bx_n(1 - x_n) \quad (2)$$

where y_n represents the ratio of arms expenditure to Gross National Product (GNP) in country A (say the Soviet Union) in the year n , and x_{n+1} represents the same ratio for country B (say the United States) in the following year. The stability of the arms race is predicted from the disposable parameters a and b .

According to these equations, as the expenditures of nation A approach its GNP, those of nation B approach zero. This does not seem reasonable. A kind of saturation term might be preferable, but would probably not produce the "chaotic" behaviour desired.

Although the ideas are linked semantically, the chaos of warfare and of fluid dynamics are otherwise dissimilar. In warfare, the variables representing arms expenditures do not undergo a transition to a wandering unpredictable distribution, as the equations imply. Predictably, they will show a dramatic increase.

In his examples of various arms races, Saperstein gives data for only two years. For the Soviet Union and the United States, data are available² for the 10-year span 1971 to 1980 (see table below). To compute a least squares estimate for the parameter b requires minimizing the function

$$f(4b) = \sum_{i=1}^{n-1} [4bx_i(1 - x_i) - y_{i+1}]^2 \quad (3)$$

Setting $f'(4b) = 0$, the function has a minimum at

$$4b = \frac{\sum_{i=1}^{n-1} [x_i(1 - x_i)(y_{i+1})]}{\sum_{i=1}^{n-1} [x_i(1 - x_i)]^2} \quad (4)$$

This gives $4b = 2.55$, or $b = 0.64$ (compared with 0.54 as estimated by Saperstein.) Similarly, $4a = 0.467$, or $a = 0.12$ (compared with 0.15). After transforming

the variables in the nonlinear equations, linear regression can be performed:

$$y_{i+1} = 4bX_i, \text{ where } X_i = x_i(1 - x_i) \quad (5)$$

and

$$x_{i+1} = 4aY_i, \text{ where } Y_i = y_i(1 - y_i) \quad (6)$$

Equation (5) represents the prediction of Soviet expenditures based on previous US expenditures, and equation (6) the reverse.

The correlation coefficient measuring agreement between the model and the actual data is given by

$$r^2 = SS_{\text{regression}} / (SS_{\text{regression}} + SS_{\text{residuals}}) \quad (7)$$

where

$$SS_{\text{regression}} = \sum_{i=1}^{n-1} (4bX_i - \bar{y})^2, \quad (8)$$

$$\bar{y} = 1/(n-1) \sum_{i=2}^n (y_i) \quad (9)$$

and

$$SS_{\text{residuals}} = \sum_{i=1}^{n-1} (y_{i+1} - 4bX_i)^2 \quad (10)$$

For the prediction of Soviet expenditures from those of the United States, $r^2 = 0.518$. Analogously, the prediction of US expenditures from those of the Soviet Union gives $r^2 = 0.130$. A plot of the residuals demonstrates a clear trend, indicating a systematic error in the model.

For comparison, Hamblin³ obtains correlation coefficients better than 0.9 in applying his curvilinear model to arms expenditures before the First World War, to "warlike worktime" before the Second World War and to satellite launchings, ballistic missile production and nuclear explosions in more recent times. These data sets are more accurate than the proportion of GNP devoted to arms, which rests on many assumptions. The asymmetry of Hamblin's model (in contrast to Saperstein's) has serious policy implications: he assumes one party to the arms race to be the leader.

While Hamblin³ and Richardson⁴ discuss the possible meaning of their parameters, Saperstein unfortunately does not speculate on what might influence the values of a and b , and hence the stability of the arms race. We share his concern that we may be closer to the threshold of war than the model suggests, especially considering the magnitude of actual expenditures rather than the theoretical parameters. The proportion of resources devoted by the

Soviet Union to arms has been matched in peacetime only by that of Nazi Germany just before the Second World War.

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Basement membranes and epithelia

SIR — There is considerable confusion over the nomenclature of "basement membranes" of epithelia. We propose, in accordance with usage in a number of leading laboratories, to restrict the term *basement membrane* to the (usually) fibrillar layer produced by the connective tissue fibroblasts, and to use the term *basal lamina* only for the acellular glycocalyx immediately underlying the epithelial cells. The basement membrane can always be resolved by light microscopy, the basal lamina as a rule only by electron microscopy. The distinction is a morphological and operational one and as such is perfectly unambiguous. (We leave aside the question whether the basal lamina may be regarded as part of the basement membrane.)

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Gene conversions and crossing-over

SIR — Fink and Petes¹, in their News and Views commentary on the interesting papers by Klein² and by Klar and Strathern³, refer repeatedly to the "50 per cent rule" relating gene conversion to crossing over and state that this rule has been so compelling as to have been "incorporated into virtually every current model of recombination". This may give a wrong impression. As Klein points out, it has long been known that intragenic recombination at meiosis within certain genes in various eukaryotes may involve crossing over far less than 50 per cent of the time. An association of as low as 10 to 30 per cent is not at all unusual in *Sordaria*⁴, *Neurospora*⁵ and *Drosophila*⁶. Even in *Saccharomyces*, as Stadler pointed out in his 1973 review⁷, the figure comes down to 35 to 40 per cent when proper allowance is made for coincidental crossovers.

It would be remarkable if virtually every current model really were committed to a 50 per cent rule, and in fact this is not so.

Proportion of GNP devoted to arms expenditure

Year	United States x_i	Soviet Union y_i	Year	United States x_i	Soviet Union y_i
1971	0.070	0.144	1976	0.053	0.134
1972	0.066	0.147	1977	0.053	0.131
1973	0.060	0.142	1978	0.051	0.142
1974	0.061	0.142	1979	0.051	0.143
1975	0.059	0.144	1980	0.055	0.146

The model proposed in 1975 by Meselson and Radding⁸, which was put forward for quite another good reason (the need to reconcile the Holliday model with the increasingly strong evidence for hybrid DNA on one participating chromatid only), provides a very plausible way of uncoupling conversion from crossing-over to any desired extent. According to this model, the first step leading to gene conversion is the unilateral invasion of one participating chromatid (DNA duplex) by a single DNA strand unwound from the other. The reciprocal strand-crossing and the formation of the symmetrical cross-strand structure (Holliday junction), which hypothetically sets up the possibility of crossing over, follows later after a series of manoeuvres that could easily require special conditions (such as may prevail more or less during meiosis) for their efficient performance. An apparently easy alternative is for the initial single bridge to be cut by an endonuclease, the invading single-strand fragment ligated into the recipient chromatid and the gap in the donor chromatid filled by repair synthesis. This could lead to conversion without any possibility of crossing over.

Since crossing over (both in fact and in theory) can be so easily uncoupled from conversion even in regular meiosis, it is no surprise to find it largely avoided during genetic exchange between repetitive sequences at non-homologous loci, when its occurrence would lead to highly deleterious chromosome rearrangements. But perhaps it is not avoided completely. Spontaneous chromosome aberrations do occur at a low frequency, and something like a "0.1 per cent rule" governing interactions between dispersed repeats could account for many of these.

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SIR — Fink and Petes¹ in writing on gene conversion make repeated reference to a so-called 50 per cent rule. According to this, half the intragenic conversion events in fungi are associated with reciprocal recombination. These authors imply that there is compelling evidence for this conclusion. In reality there is much variation in the frequency with which aberrant segregation is associated with reciprocal exchange, with values ranging from 15 per cent to 75 per cent (see ref. 2, p. 321). Only one example seems to be known of a satisfactory fit with 50 per cent, namely, aberrant 4:4 segregator at the *g* spore

colour locus in *Sordaria fimicola*^{3,4} (and see ref. 2, p. 240).

Klar and Strathern⁵ have discovered that conversion involved in yeast mating type switching during the mitotic cycle is not associated with reciprocal exchange and Klein⁶ has made the same discovery for meiotic intrachromosomal conversion between duplicated inverted sequences at the HIS3 locus, also in *Saccharomyces cerevisiae*. Fink and Petes¹, in commenting on these results, overlook the evidence of various kinds from organisms as diverse as *Drosophila melanogaster*^{7,8}, *Secale cereale* (rye)⁹ and several fungi¹⁰⁻¹³, that eukaryote recombination may be a two-step process, with gene conversion events in the first step and reciprocal exchange in the second (see ref. 2, pp. 324, 336, 343). The results obtained by Klar and Strathern⁵ and Klein⁶ could then be accounted for if intergenic recombination differs from the normal process in lacking the second step.

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Snookered by colour differences

SIR — Your correspondent William Hickmott (*Nature* **310** 731-732 1984) reported a hitherto unrecognized property of colour. My own part-time socio-physiologico-behavioural research, carried out over many years, lends further weight (*sic*) to the hypothesis proposed, although my extensive data, collected for a different purpose, reveal a different ordering. My research has been on the influence of the recreational context on the liquid consumption of the adult human male.

From the mass (*sic*) of data which I have accumulated I extracted the small subset relating to a particular recreational activity in which pairs of males use sticks to strike small spheres of identical size on a large green rectangular surface. Naturally, in order to minimize the "Hawthorne" effect I have always observed from a great distance (~10³ cm) and have never enquired as to their purpose. (I opine that they are amateur physicists constantly testing and retesting Newton's laws of motion. Their efforts are, however, constantly frustrated since the experimental surface has a number of

roughly circular defects into which spheres frequently disappear.)

All this brings me to the point of my observations. The spheres are of different colours, presumably to facilitate their differentiation in the heavily polluted atmosphere in which these experiments usually take place. The behaviour of these spheres is most curious and for a long time the reason eluded me, but it can be explained now by your correspondent's hypothesis.

The red spheres behave most oddly of all — when they disappear down a defect in the experimental artefact they are not reused, unlike the other coloured spheres. (This correlates well with your correspondent's observations of the red tomato, and suggests that the effect of this colour is so great that the objects are more easily damaged.) As the red spheres disappear the effect of the other coloured spheres is more easily discernible, although the effects are exceedingly complex.

My computer analysis of the results has led me to the inescapable conclusion that the colours do indeed display action at a distance. The disappearance of the colours, usually temporary, triggers a behavioural response in the males which manifests itself in the physical moving of pointers on a scale displayed on the wall near the experiment. I have no idea of the function of these scales, but they may be of symbolic or ritualistic significance.

From my analysis (Imperial undimensional scaling), I conclude that the order of the colours is: red, yellow, green, brown, blue, pink and black, with white occupying an anomalous position on a dimension normal to this. This seems to be almost completely the opposite of the ordering which your correspondent reported, but this need not cause us to reject his basic hypothesis.

The answer clearly lies in the observations discussed by John Maddox (*Nature* **310**, 723; 1984) — the inverse square law needs to be generalized to an inverse sphere law in the complex situation where more than two bodies are involved. As a corollary, the colour of the spheres in the experiments to measure *G* is probably the reason for the variations observed.

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where Matters Arising remains appropriate).

Forest succession

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The once-standard holistic theory of forest succession has more recently been overtaken by a Neodarwinian reductionist theory with defects of its own. A less inflexible theory is much needed.

THE term succession is used to describe many types of vegetation change on widely different scales in both space and time. In discussing forested or potentially forested zones, succession is formally defined here as the directional change with time of the species composition and vegetation physiognomy of a single site where climate remains effectively constant. Understanding succession is important for two reasons—the value of the concept in the development of ecology as a science and its enormous potential in the development of programmes for the conservation and exploitation of biological resources^{1,2}. Succession is nevertheless a most controversial issue, involving confrontation between holism (the view emphasizing unity and integration in nature), and reductionism (in which chance and darwinian interpretations dominate).

Primary successions occur on previously unvegetated terrain. Glacial moraines in Alaska, for example, eventually support spruce forest after first being colonized by herbaceous plants, which are succeeded by thickets of willow and alder. Secondary successions occur on disturbed areas, including those converted to agriculture and subsequently abandoned. In parts of the east coast of the United States, the herbaceous communities of such 'old fields' are quickly invaded by pines and then by oak, hickory and other species characteristic of the original forests of the region. Environmental changes occurring during succession are classed as autogenic when they are a consequence of the presence of plants. Examples are the accumulation of organic matter in soil and the modification of light levels within vegetation. Allogenic changes, on the other hand, are those caused by factors external to the vegetation. Examples include the deposition of silt by rivers and local temperature changes as glaciers retreat.

No direct study of forest succession has so far been possible because of the longevity of the organisms involved. Descriptions of succession are usually inferred from studies of the vegetation of adjacent sites of different known ages, in which case it is necessary to assume that sites differ only in age and not in substrate material, climatic history, past disturbance or the input of propagules. Although often reasonable, the validity of this assumption is not always carefully assessed³; descriptive field studies rarely provide much insight into the mechanisms of successional change, which remain hypothetical and are a major concern of the long-standing theoretical controversy over succession.

Theories of succession

The development and interrelationships of holistic and reductionist theories have been reviewed by McIntosh^{4,5} and these are briefly summarized and contrasted here. Common to both theories, however, is the natural history of trees. The broadest distinction that can be made between tree species participating in successions is between 'pioneer' trees, those species usually most prominent on open sites and absent from forest, and 'forest' trees, individuals of which contribute to the canopy of mature

forest. The classification^{6,7}, summarized in Table 1, may be a 'crude oversimplification' (see ref. 8), but it is necessary for presentation of the general arguments on succession⁹.

Holistic ecology takes the ecosystem, a unit of environment and biota in reciprocal interaction¹⁰, as its object of study. Ecosystems are held to possess 'emergent' properties—properties which cannot be inferred from those of the parts of the system. Successional change is often considered to occur in stages through wave-like invasions by groups of species, and is thought to be controlled by the vegetation itself. Thus autogenic change, caused by the presence of a particular species or group of species, makes the environment suitable for the next group of species and so facilitates successional change. This is the 'facilitation' hypothesis. In holistic theory, the development during succession of 'biological control' of nutrient cycling is also emphasized. Succession is interpreted teleologically as the process of development of an ecosystem of maximum stability (by the yardstick of resistance to disturbance) and of maximum efficiency in the utilization of resources¹⁰. Successional change is usually held to be orderly, predictable and therefore deterministic, converging to forest from various different starting points.

Table 1 Empirically determined attributes of pioneer and mature forest tree species

Characteristic	Pioneer tree	Forest tree
Dispersibility of seed	To long distances wind/bird/bat	Short distance rodent/bird/none
Seed weight	Light to heavy	Relatively heavy
Seed germination: light-stimulated	Yes	No
inhibited by far red light	Yes	No
Longevity of individual	Shorter	Longer
Time to repro- ductive maturity	Shorter	Longer
Height growth	Fast	Slow
Height at maturity	Shorter	Taller
Resource acqui- sition rates	Fast	Slow
Photosynthesis light-saturated at	High light intensities	Low light intensities
Recovery from resource limitation	Fast	Slow

These categories represent the extremes of a spectrum of ecologies; pioneer is analogous to 'r-selected', forest to 'k-selected'. Reductionist authors assert that these differences between pioneer and mature forest species explain all the phenomena of succession (see also Fig. 1) (from refs 7 and 8).

In the past decade, the defects and lack of applicability of holistic theory have been convincingly exposed and a new body of reductionist theory has developed^{11–16} based on Gleason's interpretation of the plant community as a fortuitous assemblage of species populations, each with a unique behaviour¹⁷. The fundamental stochasticism of this view is supported by evidence of several alternative successional pathways within a given vegetation type¹⁸. The cornerstone of the new successional theory is the modern interpretation of Egler's¹⁹ initial floristic composition (IFC) model (Fig. 1). In this model all the species participating in a succession become established at or very shortly after initiation. Succession is merely the sequential physiognomic dominance of the site by species with different life histories, growth rates and sizes at maturity. The facilitation hypothesis is rejected, and autogenic changes are seen as neutral or inhibitory factors rather than the driving force of succession. The theory emphasizes that the availability of resources may decrease with succession as the density of individuals increases. Pioneers are seen as opportunists exploiting environments free from 'competition', while forest trees are efficient and conservative in their use of resources. This interpretation of autogenesis has resolved into two subsidiary models, 'tolerance' and 'inhibition', considered more fully below. Although a reductionist, darwinian approach^{20,21} has given great impetus to contemporary plant ecology, enjoying the heuristic advantages inherent to reductionism, the new successional theory has developed independently of this mainstream and rests not on new empirical findings, but on reinterpretations of existing data. Unease has already been expressed at this development^{4,5,22}; is there progress if models such as IFC, tolerance and inhibition simply replace the holistic framework as the dogma by which inadequate field data are interpreted?

This review is a critical appraisal of the theory as it now stands, and suggests a better approach in considering succession. The ideas developed in references 11–18 will be referred to as the 'reductionistic approach' and their authors as 'the reductionists'.

Reductionist succession

Tolerance is the ability of individuals (particularly of juveniles) to survive in the poor environmental conditions resulting from the high density of other individuals. Foresters have long been aware that tree species differ in their ability to become established and grow within a forest^{23,24}. Although this may be due in part to factors affecting the availability of soil moisture and mineral nutrients, tolerance has come to be synonymous, in successional theory, with the ability to persist or grow in shade. By this definition, pioneer species are intolerant and forest species tolerant, because pioneer trees cast shade, thereby preventing their own regeneration and are replaced by forest trees, which can become established in the shade. It is well known that felling stands of intolerant pines on old fields increases the growth of the tolerant juvenile forest trees in the understorey, raising the suggestion that autogenic change (shading) delays rather than facilitates succession¹¹. Unfortunately, however, removing the understorey also increases the growth of pines²⁵, introducing the first constraint on this simple model.

Some have nevertheless unacceptably adopted tolerance as the sole mechanism of forest succession^{26,27}. Tolerance is clearly an essential component of the biology of forest trees. However, it being the only mechanism of succession depends on whether environmental factors other than quantum flux are limiting to establishment and growth, whether the states of these factors change as succession proceeds and whether pioneer and forest species differ in their responses to these environmental changes. At the outset, the possibility must at least be considered that juveniles of some forest trees not only tolerate the environment of a pioneer forest canopy, but require it. Silvicultural experiments in the rain forests of Malaysia, for example, have shown a wide variation between species in the responses of apparently suppressed saplings to the clearance of canopy trees⁸. The growth of some species accelerates, as the tolerance model

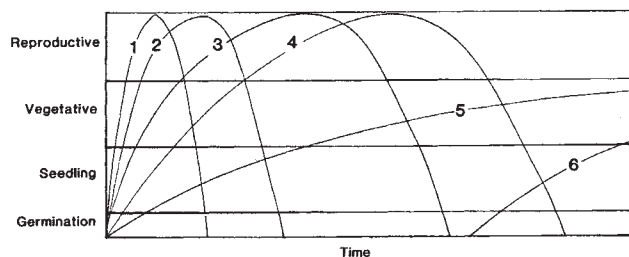


Fig. 1 Schematic representation of the initial floristic composition model of succession. Species 1–5 become established at the initiation of the succession, and successively dominate the site due to the different lengths of time they take to reach maturity. Species 1 may represent an annual herb, species 4 a short-lived shrub, and species 5 a slow-growing, shade-tolerant forest tree. Species 1–4 fail to regenerate as they are light-demanding. Late colonization by species 6 occurs as a consequence of the release of the inhibitory effects upon it of species 3 (modified from Gómez-Pompa and Vásquez-Yanes³⁹).

predicts, but individuals of other species become stunted, susceptible to disease or predation, or are killed.

The inhibition model proposes that the vegetation occupying a site prevents the invasion of other species. The best evidence for this model is the demonstration of apparent long-term stability in certain vegetation types, mainly monospecific stands of clonally spreading shrubs¹³. These areas may not be typical; they are usually small or extremely small, and sometimes have an unusual management history. Thus inhibition may not be a general phenomenon in forest succession; more work is required to establish the frequency with which it occurs.

Initial floristic composition

Initial floristic composition (IFC) has become accepted as a descriptive model for both primary^{4,16} and secondary successions¹¹. Although this model fits field observations more closely than did holistic ideas, it is certainly not an explanation, nor even a general description, of succession. Niering and Goodwin suggest¹³ that, with respect to North American old field successions, IFC requires 'conceptual elaboration' and go on to include the supposedly more general cases in which woody species are not present at the initiation of successions, but appear within a few years. This elaboration neither describes nor explains the behaviour of many forest tree species.

There is a substantial body of information about old field successions in several forest regions of the United States. Two groups of woody species consistently appear within 10 years of the abandonment of a field. The first comprises short-lived shrubs and small trees, and is exemplified by the species of *Prunus* and *Juniperus* recorded on recently abandoned fields in New Jersey^{28–30}. The second group consists of larger and longer-lived trees which may also occur as components of mature forest. In the Carolinas and southern Virginia, this group includes loblolly pine (*Pinus taeda*) and shortleaf pine (*Pinus echinata*), which may appear in large numbers after one year, the density of seedlings depending on the proximity of parent trees^{31,32}. Red maple (*Acer rubrum*) behaves similarly²⁹. Despite the claims of Niering and Goodwin¹³ and others, it is clear that almost all the oaks (*Quercus* spp.) and hickories (*Carya* spp.) characteristic of the mature forests of many of the central and eastern states of the United States are significantly later colonizers of old fields. Colonization by oaks has been recorded between 11 and 20 years after the abandonment of fields^{28–36}, except in two cases in which very early colonization was induced by the presence of overhanging parent trees^{37,38}. Longer periods of time elapse before the appearance of hickories; sites abandoned for 40 and 60 years are amongst the most recent from which new individuals have been reported^{33,34}. (The one exception is a record of hickory

seedlings after three years on one of the 10 sites monitored over 11 years by Buell *et al.*²⁸, but further colonization has not been reported during this continuing study³⁰.) In all the cases cited, colonization by oaks and hickories occurred on sites already supporting established pioneer vegetation.

Studies of secondary forest succession in the humid tropics have been ignored by the reductionists. The 'old fields' of the tropics are forest clearings abandoned by farmers practising shifting cultivation. It is generally accepted that species of late successional and mature forests do not colonize such clearings until there is already a well established pioneer vegetation, dominated by fast growing pioneer trees^{2,8,9,39-41}.

In England, primary successions in abandoned quarries recently received some attention in a study of the vegetation of three chalk quarries⁴². Birch (*Betula pendula*) and willows (*Salix* spp.) are able to behave as pioneers on bare chalk, while hawthorn (*Crataegus monogyna*) and English oak (*Quercus robur*) are clearly later colonizers, appearing once the sites already support pioneer scrub (Fig. 2). A survey of 48 chalk and limestone quarries throughout the country suggests that this pattern of colonization has been repeated several times⁴³.

On many sites undergoing succession, the seedlings of shrub and tree species such as *Crataegus* and *Prunus* spp., and the tropical genus *Cecropia*, exhibit markedly clumped distributions, occurring mostly under the canopies of established trees^{42,44-49} (see Fig. 3). These species may be very early colonizers of abandoned sites, such as orchards which already support trees^{44,45}, or later colonizers in primary successions, such as those occurring in abandoned chalk quarries⁴².

Different tree species seem not to have become established simultaneously at the initiation of any of the successions described here, so that the simulative and predictive power of IFC would appear to be limited. Differential seed dispersal is probably chiefly responsible for the failure of this model, but this does not imply that it is sufficient to regard dispersal simply as a stochastic process in which light wind-dispersed seeds will usually go further in a shorter time. The role of animal dispersal vectors will be crucial to the colonization of successional sites by many woody species. Of the pioneer trees mentioned, the pines, maple, birch and willow all have wind-dispersed seeds⁵⁰, while those of tropical forest pioneer trees are widely dispersed by birds and bats^{51,52}. Oaks, hickories, other temperate forest species such as beeches, and many of the trees of undisturbed humid tropical forests have large short-lived seeds^{50,53,54} which lack the fleshy pulp and other characteristics of seeds specifically attractive to dispersal vectors, but which are nevertheless dispersed by the hoarding behaviour of forest rodents and birds⁵⁵. It is probable that many such forest vertebrates do not enter open fields or clearings except on brief forays from surrounding hedgerows or forest, as in the case of the red squirrel (*Tamiasciurus hudsonicus loquax*)⁵⁶. Acorns are a major component of the diet of the jay (*Garrulus glandularius*), which buries them in forest, open woodland or around isolated trees, but avoids open fields^{57,58}. The genera *Crataegus*, *Prunus* and *Cecropia* all bear fleshy fruits, dispersed by frugivorous birds (and bats, in the case of the tropical taxon). It is likely that in all these cases, colonization of a site requires the presence of trees in which the dispersal vectors may roost^{42,44-49}; similar considerations may apply for the dispersal of the many species of canopy trees in humid tropical forest.

These observations are consistent with the hypothesis that the presence of pioneer woody species often increases the rate of colonization of a site by other woody species whose seeds are dispersed by animals and birds since the pioneers render the site more attractive to dispersal vectors (see also ref. 59). The behaviour of the vectors is probably decisive; bird-dispersed shrubs do themselves behave as pioneers on old fields in the United States^{28-30,60} and in England⁶¹. The testing of this hypothesis would be welcome.

The few known successions which actually resemble IFC are of two types. The first is where forest species with wind- or ballistically-dispersed seeds dominate the local flora, as in the

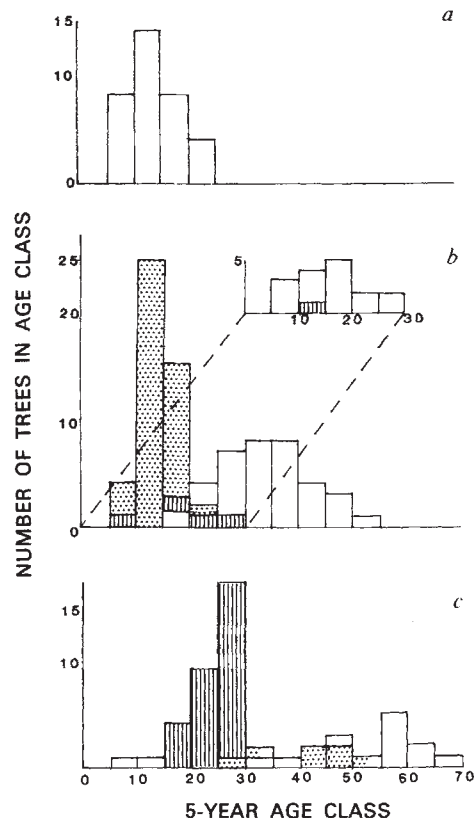


Fig. 2 Succession in abandoned chalk quarries in southeastern England. Histograms represent the age structures (excluding first year seedlings) of populations of birch (*Betula pendula*, open bars), oak (*Quercus robur*, vertical lines) and hawthorn (*Crataegus monogyna*, stippled) in representative stands on the three adjacent quarries, in August 1982. Vegetation was homogenous at the scale sampled, removing species/area effects. a, Site abandoned for approximately 7 years; b, site abandoned for ~35 years; c, site abandoned for ~60 years. Birch is a primary colonizer, appearing before abandonment of the sites. Oak and hawthorn are much later colonizers; oaks in c were well formed, some attaining the height of the oldest birches. Seedlings of hawthorn were recorded in a in 1982 (Fig. 3). The inset in b records another part of this site and illustrates spatial variation in colonization. Although these data show populations each with an individualistic behaviour, they do not conform to the IFC model.

upland evergreen forests of Ghana⁶², on mudflows in Californian river valleys⁶³, and on Alaskan glacial moraines. (Other factors affecting moraine successions, however, would seem to limit the value of IFC in even this case, as will be shown below.) The second is the regrowth of forest after clear-cutting, when the succession is dominated by pioneer trees from viable seeds already present in the soil and the regrowth of forest species from stumps⁶.

The role of autogenic change

Implicit in the reductionistic approach is the assumption that different tree species are equally able to become established and develop normally in the environment of a newly available site. The reductionists, however, carefully do not analyse contradictory evidence, probably because it leads towards the facilitation model. Drury and Nisbet cite undeniable evidence for the role of allogenic change¹¹, but their attempt to refute the hypothesis that autogenic soil development plays a role in primary succession does not succeed. Botkin emphatically concludes that the elements of the reductionistic framework (as defined here) are sufficient to account for all the phenomena of succession⁶⁴. His computer simulations reproduce the growth of established individuals of different tree species on mature soils, so that it

is scarcely surprising that environmental changes other than shading were found to be unimportant. A large body of observations made in other circumstances remains to be incorporated into the reductionistic framework. Part of the difficulty is that the evidence is scattered in a wide range of sources, and is not always directly related to succession. This is unavoidable in a field where progress towards understanding the mechanisms at work has only just begun⁶⁵.

Harper provides a basis for understanding the recruitment of seedlings²¹. A germinating seed occupies a 'safe site', a microenvironment which provides, for example, dormancy-breaking stimuli, the resources necessary for germination (particularly water and oxygen) and freedom from predators and pathogens. Microenvironmental heterogeneity can have dramatic effects on seed germinations which varies between species in a manner related to the different sizes and shapes of the seeds. Environmental factors affecting the survival of seedlings include the availability of moisture, temperature variations and predators. All of these factors may be modified by the presence of neighbouring plants, and each is subject to autogenic change during succession. The accumulation of organic matter in the surface layers of the soil during forest succession on old fields and the floors of abandoned chalk quarries increases the retention of moisture in the zones most affecting seeds and seedlings^{32,42,66}; this is probably a feature of all successions in which organic soil horizons develop. In the humid tropics, diurnal soil and air temperatures and saturation deficits of the air, are all lower in forests than in open clearings, largely because of shading⁶⁷⁻⁶⁹. Indeed, such spatial environmental heterogeneity is a general feature of forest microclimates⁷⁰, and the kind of environmental changes to be expected during succession can be extrapolated from these findings. Pioneer and forest species may, however, differ in their responses to these autogenic changes, as will now be examined.

Generalizations are not possible from studies of the environmental requirements and tolerances of pioneer trees colonizing clear-felled forest sites where removal of forest trees is assumed to increase the availability of water and mineral nutrients. The pioneer trees of primary successions do not enter an environment with the freely available resources of clear-felled forests; neither, it would seem in some cases, do those colonizing old fields.

Within young, primary successional birch woodland (Fig. 2, site b), the mortality of seedlings of birch and willow is higher on bare chalk than in gaps in a turf of mosses and herbaceous plants⁴². In a neotropical secondary succession, the recruitment on bare soil of seedlings of the pioneer tree *Cecropia filicifolia* was markedly increased by treatments which reduced surface temperatures and evaporation⁷¹. It seems certain that tolerance of periodic drought and other environmental extremes is a critical factor in the survival on old fields of pioneer loblolly pine, red cedar (*Juniperus virginiana*) and elm (*Ulmus alata*)^{72,73}. (An important component of the biology of seedlings of pioneer species is that they should be tolerant of, or have a mechanism to avoid, high leaf temperatures induced by short-wave solar radiation at exposed sites⁷⁴.) Examples of sites which become dominated by pioneer species suggest that this occurs by toleration, not by exploitation of the environment.

There are very few critical studies on the establishment of forest trees in pioneer environments but there is much evidence to suggest that forest species do not have the ability of pioneers to become established there. Acorns are readily killed by heat, desiccation or winter frost when surface-sown on open sites⁷⁵⁻⁷⁷. Germination requires burial, to protect acorns against environmental extremes, to facilitate uptake of water and to protect them from seed predators, a major cause of mortality⁷⁶. These characteristics are probably shared by all large tree seeds, providing the first potential barrier to establishment on open fields and clearings in temperate and tropical zones.

The next potential barrier may occur at the seedling stage since seedlings of red and white oaks (*Quercus rubra* and *Q. alba*) are intolerant of drought and do not survive on drier forest sites⁷⁸; survival of red oak seedlings planted on coal spoil

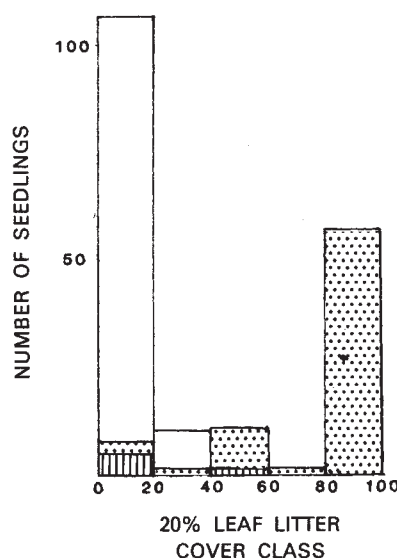


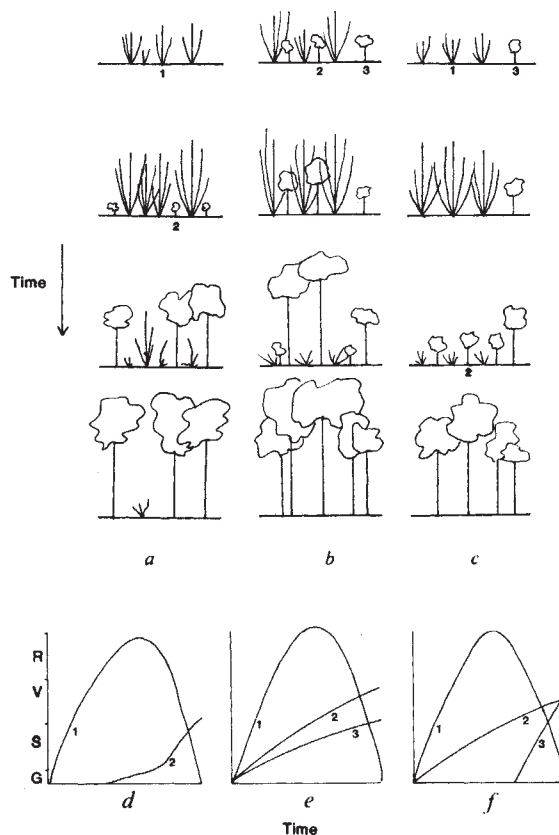
Fig. 3 Separation of seedlings of three tree species along a spatial gradient in ground cover of leaf litter in site a of Fig. 2, September 1982. Data are summarized from 60 quadrats of 0.25 m², 10 randomly positioned in each of six 25-m² stands of birth/willow scrub. All quadrats in the 81-100% litter cover class were directly under the canopies of willow bushes. The seeds of birch (vertical lines) and willow (open bars) are widely dispersed by wind, but bare ground is required for the establishment of seedlings of these species. The distribution of hawthorn seedlings (stippled bars) is probably a consequence of patterns of seed deposition by frugivorous birds perching in established bushes.

is greatly improved by treatments reducing the high surface temperatures and the incidence of moisture deficits⁷⁴. On a Michigan old field, seedlings of *Quercus velutina* and *Q. alba* survived when established among herbaceous vegetation, but not on bare ground⁷⁹. Even within forest, seedlings of beech (*Fagus grandifolia*) occur much less frequently on dry south- and west-facing slopes than on those facing north and east⁸⁰. European beech (*F. sylvatica*) does not establish from seed on open ground⁸¹; *Agathis australis* in New Zealand requires the presence of associated shrubs⁸². Shade may be required for the successful establishment of seedlings of tropical forest trees of the family Dipterocarpaceae⁸, possibly as a consequence of intolerance of drought and high saturation deficits, and of the high leaf temperatures likely to be experienced in full sunlight.

Traditional theories assign autogenic development of soil and mineral nutrient cycling a functional role in two types of forest succession—secondary successions on abandoned shifting cultivation clearings in humid tropical forests, and primary successions on substrates such as glacial moraine. In the former type, forest clearings are abandoned when they will no longer produce a useful crop, partly because of loss of soil fertility. The ecological implications of this have been discussed². After abandonment, the site is often invaded by pioneer trees and improvements in soil fertility take place, which has been demonstrated on sites in Nigeria and Ghana^{83,84}. Sites where such an increase in soil fertility does not occur, such as are found in Costa Rica⁸⁵ where the soils are unusually fertile⁸⁶, may well be atypical. Extrapolating from Jordan and Herrera's⁸⁶ conclusions, it is possible that in the humid tropics facilitation may operate in successions on less fertile soils such as those of the Amazon basin, which are intensely leached. Much work remains to be done to resolve this issue.

The major early constraints on plant growth during primary succession are common to all environments; a lack of available macronutrients such as nitrogen and phosphorus and a lack of soil organic matter^{42,59,87-98}. In physically extreme environ-

Fig. 4 Composite mechanisms of succession. The tolerance model operates in all examples, as light-demanding pioneer trees fail to regenerate. Inhibition, tolerance and facilitation affect the colonization and development of forest trees. *a*, Seed dispersal vectors of a large seeded forest tree do not enter a site until pioneer vegetation has developed sufficiently to provide a habitat attractive to them. The seeds germinate but the seedlings do not develop in the environment of the pioneer vegetation; the seedlings are, however, protected from the potentially deleterious effects of direct sunlight. The seedlings develop to maturity on the deaths of individual pioneers. Facilitation operates at the seed dispersal stage, tolerance and facilitation simultaneously at the seedling stage. *b*, A nitrogen-fixing pioneer and a forest species become established simultaneously on an extremely infertile mineral substrate. Shade-tolerant juvenile forest trees in the pioneer thicket benefit from the nitrogen fixation and other environmental changes consequent on the presence of the pioneer. Open growth forest trees develop more slowly, and are overtopped both by 'nursed' individuals and by later colonizers. Facilitation and tolerance operate at the juvenile stage, although open grown forest trees may also develop to maturity. *c*, A similar situation to *b*, but forest trees are unable to become established in the pioneer thicket, colonizing the nitrogen-rich environment left after the deaths of the pioneers. Inhibition operates at the establishment stage, facilitation at the juvenile stage. *d-f*, The population patterns associated with each model. Although these are similar to IFC (Fig. 1), the actual ecological process that the diagrams describe is the outcome of more than simply differences in the life-history characteristics and shade tolerances of the species concerned. 1, Pioneer tree; 2, nursed or late colonizing forest tree; 3, open grown forest tree; vertical axis as in Fig. 1.



ments, nitrogen-fixing herbs, shrubs (for example, *Dryas drummondii*) and small trees (for example, the alders, *Alnus* spp.) occur as pioneers or very early colonizers in successions on basalts in Japan⁸⁷ and glacial moraines in Switzerland, Alaska and Canada⁸⁸⁻⁹⁸. These species must be largely responsible for the marked increases in the amounts of nitrogen and organic matter in the substrates of moraines of increasing age, the factor traditionally assumed to facilitate other species. One exception is the Yukon Territory⁹⁶⁻⁹⁸ where the short growing season is probably the dominant ecological factor.

When it was realized that floristic descriptions of primary successions often resemble IFC^{4,11}, facilitation was then held to have been rejected as an explanation of succession. The crucial point, however, is that the different models of succession are not mutually exclusive. Even where seedlings of forest trees occur in a pioneer environment, both the probability that they will survive and the rate at which they develop to maturity may be increased by continuing autogenic changes. Cooper, for example, is convinced that pioneer spruce (*Picea sitchensis*) at Glacier Bay, Alaska, were permanently stunted and overtopped by later colonizers encountering less extreme conditions⁸⁹⁻⁹¹. Other workers have described symptoms of poor growth, chronic nitrogen deficiency and the probability of high mortality in seedling and juvenile cottonwood (*Populus trichocarpa*), spruce and hemlock (*Tsuga mertensiana* and *Tsuga heterophylla*) on recent moraines⁹²⁻⁹⁴. These observations, however, do not point unambiguously to either holistic model for primary successions, and await explanation. Experimental studies on natural vegetation are clearly required; attention has, however, been drawn by Bradshaw to the possible functional similarities between the environmental changes of primary successions and those brought about artificially through land reclamation work⁹⁹ (although his generalizations about the amounts of nitrogen required in the 'ecosystem' before it will support trees remain to be substantiated). Nitrogen-fixing shrubs and trees are widely used as nurse crops for less robust species while the soil-improving properties of alders have long been appreciated¹⁰⁰. Thus *Alnus sinuata* is recommended as a nurse crop for douglas

fir (*Pseudotsuga menziesii*) on sites low in nitrogen and organic matter¹⁰¹. In New Zealand, a nurse crop of tree lupin (*Lupinus arboreus*) is essential to the establishment of *Pinus radiata* on sand dunes¹⁰².

In conclusion, the generalization that because of the absence of 'competition', early successional environments offer greater levels of resources than do forests, and the corollary that pioneer trees are exploitative and forest species conservative and tolerant of resource shortages, is not supported by the evidence. Often pioneer trees show tolerance, not of shading, but of the extreme temperatures and infertile, often drought-prone substrates of many early successional environments. Forest trees may be tolerant of 'competition', but may well not be tolerant of physically hostile sites. Indeed, there is no reason to suppose that the two types of tolerance are conferred by the same biological attributes. Pioneer trees cause environmental changes which may alleviate constraints on the establishment of forest trees in initially adverse environments. But such a hypothesis and the elements of the reductionistic framework are not mutually exclusive.

The third alternative

The need for a darwinian approach to the analysis of forest succession is beyond dispute. Neither reductionist nor holistic theories of succession have produced models which explain field observations. Yet because the reductionist framework has been described in terms of the Neo-darwinian evolutionary synthesis, it tends to be uncritically accepted. As Gould¹⁰³ has pointed out, the urge to tell (and to believe) a good story affects not only holists.

In essence, the reductionists hold that succession occurs because of the existence of a spectrum of types of life history, which is the basis of the IFC model. Autogenic change is allowed a subsidiary role when its effects are seen as 'negative', as in tolerance and inhibition models. Much importance is also assigned to stochasticism, a disorganizing power as elusive as the emergent properties of the holists¹⁰⁴. The theme of this article has been that the reductionistic story fails, as it stands, for two

reasons: it neglects the nature of seed dispersal, and its generalizations about environmental change and exploitative and conservative species are untenable.

A third, synthetical approach should be sought, if only as a stimulus to further investigations. Life histories and tolerance will be important factors in any model of forest succession, but they cannot be assumed to provide a complete explanation of the phenomenon. In considering which other mechanisms are involved, it is not necessary to view facilitation as inseparable from the other elements of holism, particularly its teleological character. It is in fact adequate to suppose that the fitness of a pioneer tree is dependent only on its ability to colonize, grow and produce seed in early successional environments. The consequent environmental changes and the effect on other species are secondary results of this process. The facilitation model should allow that certain autogenic environmental changes may increase the input of seeds, the establishment of seedlings or the growth and survival of established individuals of forest species. Facilitation, tolerance, inhibition and allogeneses are interdependent mechanisms in succession and may affect the same individual successively or simultaneously during its life cycle (Fig. 4; refs 89–91). The relative importance of the various

mechanisms is likely to vary widely between environments, but attempts to classify succession on the basis of a single underlying mechanism only obscure understanding and inhibit progress.

Reductionism has not solved the succession problem, and will not do so while it relies on the same deduction from inadequate general models which led to the fall of the holistic scheme. For progress, the induction of general theory from fieldwork based in the observational and experimental techniques of plant population biology^{20,21} would seem to have much to recommend it (see ref. 105; unfortunately, not a study concerned with succession). This recalls the problem of time-scale, although many of the problems outlined above could be solved by relatively short-term studies. However, such an approach has the great advantage of theoretical agnosticism and is the least value-laden available. Any other way forward is difficult to see; the general models that have been freely proposed so far are wholly inappropriate in relation to the quality and quantity of available data.

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A model for the evolution of the Grampian tract in the early Caledonides and Appalachians

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Analogy with models developed for Newfoundland suggests that the ophiolites of the Grampian tract were emplaced as hot, young oceanic crust. The conversion of a mid-ocean ridge-fracture zone system into a subduction zone resulted in the northward emplacement of a giant ophiolite nappe onto the continental margin in the early Ordovician. This obduction occurred long before the collision between the Laurentian and Baltic Shields. The general features of this model may be characteristic of orogens resulting from oceanic closure.

OROGENIC contraction occurs in at least seven ways: (1) above subduction zones at continental margins (Andes); (2) on compressional bends of transform faults (Transverse Ranges); (3) by the compressional collapse of intracontinental extensional zones (Alps); (4) by continent-continent collision (Himalayas); (5) by arc-continent collision (Taiwan); (6) by the collision and lateral displacement of oceanic plateaus and other exotic terranes (North American Cordillera); and (7) by ophiolite obduction (Oman). The earliest interior deformations usually involved ophiolites and blueschist metamorphism, without synchronous flysch or molasse deposition in exterior zones and, therefore, probably occurred below sea level. Collapsed extensional zones have crystalline basement rocks above attenuated subcontinental mantle in the highest nappes, and lack well developed ophiolites. In orogens resulting from the closure of oceans, the highest nappes are ophiolitic, obduction preceding terminal collision

as a distinct event affecting margins. This mechanism was responsible for the evolution of the early Caledonides and is developed here.

The Grampian Orogen

There is now general agreement that the Northern Appalachian/Caledonian orogen evolved by the closing of the Iapetus Ocean¹, with terminal collision in the late Silurian/early Devonian in the Caledonides and in the mid-Devonian in the Northern Appalachians²⁻⁴. The two sides of the orogen have quite different basements, rifting and subduction histories and their only common feature was their 'stitching together' across the Iapetus Suture. The plate tectonic setting of the Grampian tract and its Appalachian equivalents (Fig. 1) has been extensively debated and hypotheses have fallen into three classes: those involving an Andean-type setting above a north-west-

Fig. 1 Simplified tectonic map of the Northern Appalachians and British Caledonides restored to the pre-Atlantic continental configuration of Bullard *et al.*⁵². Inset map shows the southwestward continuation of the Appalachians into New England. B, Betts Cove Ophiolite Complex; BA, Bergen arcs; BL, Ballantrae Ophiolite Complex; BOI, Bay of Islands Ophiolite Complex; BR, Brompton Line; C, Coney Head Complex; CB, Clew Bay Ophiolite Zone; CR, Cape Ray ophiolites; DF, Dover Fault; F, Fleur-de-Lys; G, Glover Island ophiolites; GU, Glen Urquhart; HB, Hare Bay Ophiolite Complex; HBF, Highland Boundary Fault; HK, Hamburg Klippe; IB, Inishbofin; IPS, Iapetus Suture; LA, Luke Arm Fault; LN, Lough Nafooy Fault Zone; LR, Long Range; M, Mings Bight Ophiolite Complex; MA, Mount Albert; MC, Morven/Cabrach; MQ, Macquarrie; P, Pasadena; PO, Portsoy; RF, Reach Fault; SCS, South Connemara Series; SK, Starks Knob; SUF, Southern Uplands Fault; T, Tyrone; TK, Taconic Kippe; TR, Trondheim; U, Unst Ophiolite Complex; V, Vermont ultramafic belt. [] Grenville basement within the orogen; [] Infracambrian to early Ordovician sequences in Grampian zone; [] Ordovician arc terranes; [] Ordovician/Silurian accretionary prisms; [] main ophiolite suture contact bounding Ordovician arc terranes; [] major thrusts; [] ophiolite complexes; [] oceanic and arc terranes south of the Iapetus Suture; [] Avalon/Cadomian terrane. Continuity of zones and terranes between Newfoundland and Ireland implies only tectonic equivalence and not necessarily physical continuity.

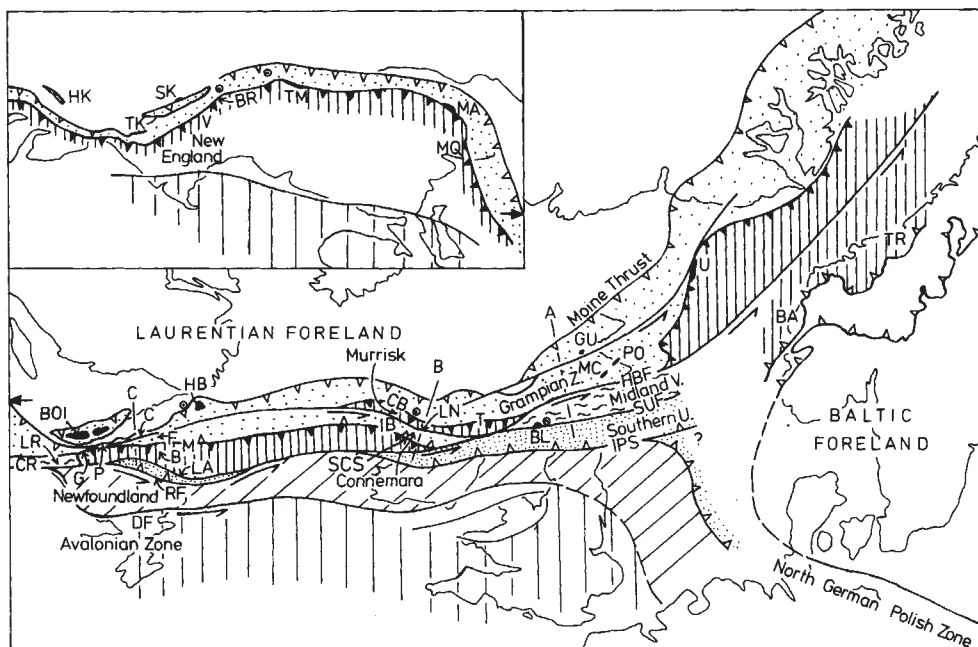
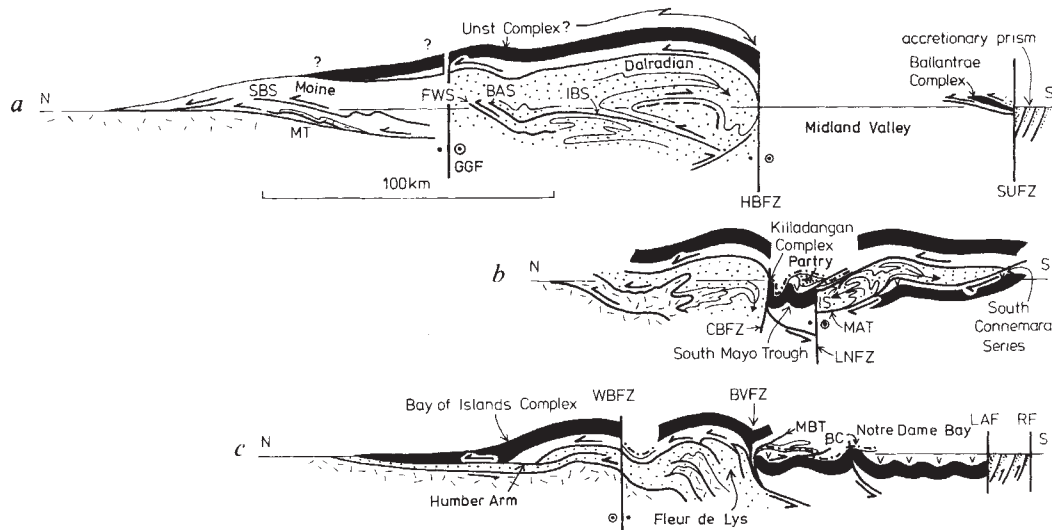


Fig. 3 Schematic structural sections (drawn to scale) along lines indicated in Fig. 1. BC, Betts Cove; BVFZ, Baie Verte Fault Zone; CBFZ, Clew Bay Fault Zone; FWS, Fort William Slide; GGF, Great Glen; HBFZ, Highland Boundary Fault Zone; IBS, Itay Boundary Slide; IS, Inisbofin Slide; LAF, Lukes Arm Fault; LNFZ, Lough Nafooy Fault Zone; MAT, Mannin Thrust; MBT, Mings Bight Thrust; MT, Moine Thrust; RF, Reach Fault; SBS, Scurr Beag Slide; SUFZ, Southern Uplands Fault Zone; WBFZ, White Bay Fault Zone. , Pre-Caledonian basement and Cambro-Ordovician shelf sequences; , Moine/Dalradian/Fleur-de-Lys sequences; white/black areas, ophiolite ultramafics/mafic; V, Ordovician arc volcanics and volcanoclastics; , Ordovician non-marine clastics; — — —, Silurian sediments.



onto the continental margin¹⁸. An Ordovician arc terrane, lying immediately to the south-east of the Grampian tract, occurs intermittently from southern New England to the British Isles (Fig. 1). In Ireland (Fig. 2g), the Ordovician arc shows a mafic to silicic maturation trend, which had begun by mid-Tremadocian times and terminated before the end of the Arenigian. In western New England, arc volcanism continued into early Caradocian times, indicating a diachronous progression of arc development, obduction and collision along-strike towards the south-west. An earliest Llanvirnian intracarbonate disconformity with karsting below and a deepening sequence into shales and easterly derived chromite-bearing turbidites above, is probably the result of flexural loading of the continental margin lithosphere²¹ by the advancing ophiolite sheet; this arching-deepening sequence is younger to the south, being from early to mid-Cardocian age in eastern New York (sub-Chazy/Balmville unconformity).

The contact between the Grampian tract and the arc terrane is a steep zone of ophiolite mélanges and south-east-facing ophiolite slivers (Brompton/Baie Verte/Mings Bight/Clew Bay/Highland Boundary Line^{17,19}), and is interpreted as the suture zone that bounds the Grampian metamorphic complex to the south-east and along which the arc terrane collided with the continental margin^{8,9,17,18}. Within this zone and an immediately adjacent tract comprising the highest Dalradian-Fleur-de-Lys stratigraphy, ophiolite mélanges and ultramafic pods are encased in a matrix of greywacke, black slates and graphitic black schists^{17,22-24} (Killadangan and Achillbeg Complexes). These mélanges are interpreted as a shear carpet formed as ophiolite-derived olistostromes overrun and deformed by the northwestward-advancing ophiolite nappe¹⁷. Similar ultramafic pods and slivers occur intermittently within the Grampian Zone in Newfoundland (Pasadena, Coney Head²⁵, Fleur-de-Lys) and in Scotland (Glen Urquhard, Portsoy) and are probably remnant slices of a single supra-Grampian ophiolite sheet tucked into the metamorphics along pinched synclinal slide zones. The Unst Ophiolite Complex is believed to occupy a southeastern root zone position analogous to the Brompton/Clew Bay Zone. The rare occurrences of blueschist in the Grampian Zone are in rocks immediately beneath the proposed ophiolite shear carpet.

Following the development of early Grampian structures with a northwestward vergence, the southeastern part of the Grampian tract and adjacent parts of the Ordovician arc terrane were affected by southward-verging retrocharriage structures following the end of arc volcanism, for example, the D4 Mannin Thrust in Connemara²⁶, the Mings Bight and southeast-verging folds and slide zones of the eastern Burlington Peninsula in New-

foundland, and the rotation of the southern margin of the Tay Nappe into downward-facing attitudes along the Highland Border. In the British Isles, they roughly coincide with the Llandelien establishment of northward subduction beneath a south-facing arc, along the southern edge of which the Southern Uplands accretionary prism developed. The southeast-verging late Grampian structures may comprise various combinations of shear associated with northward subduction, gravity spreading resulting from D1-D3 crustal thickening and rotation along fault zones at the southern edge of the rising Grampian tract. These southward-verging structures appear to be contemporaneous with, or slightly earlier than, a phase of rapid uplift and denudation of the Grampian tract that began ~460 Myr ago in the British Isles and ~450 Myr ago in Newfoundland and is evidenced by thick clastic sequences (Fig. 2j,p-s) derived from Grampian and Grenville metamorphic sources. The isotopic age pattern of the Dalradian rocks in the British Isles²⁷ indicates a period of rapid uplift, stripping and cooling from 460 to 440 Myr. Uplift was essentially completed by early Silurian times in western Ireland, where Upper Llandoveryan strata rest unconformably on the Dalradian (Fig. 2n); uplift was complete by earliest Devonian times south of the Great Glen Fault, where the Lorne lavas lie unconformably on Grampian metamorphics. Of the maximum of ~30 km that was stripped from the Grampian Highlands, 15 km was probably an ophiolite nappe; in Connemara, remnant ultramafic pods at numerous localities along the unconformity between the Dalradian and the Upper Llandoveryan suggests the stripping of ~15 km of ophiolite nappe during the mid- and late Ordovician. The Partry Series in the South Mayo Trough is the earliest Grampian molasse and, with earlier Ordovician sediments and volcanics, was folded before the Upper Llandoveryan, a phase of shortening which was probably coincident with the mid-Ordovician late D3 to D4 events affecting the Dalradian. Along the southeastern margin of the Grampian Orogen, a short but diachronous lutite lull (Glenummera Slates in western Ireland, *Nemagraptus gracilis*-*C. bicornis* black slates of Newfoundland) immediately followed arc termination and preceded uplift. Thus, we suggest, the final stages of collisional shortening led to a 'flip' in subduction polarity and the beginning of Grampian uplift and denudation.

Grampian ophiolite obduction

All giant ophiolite nappes, including those of Ordovician age in the Appalachian/Caledonian system, are characterized by a very short (<10 Myr) interval between ridge-fracture zone generation and their subsequent obduction detachment^{28,29}, have

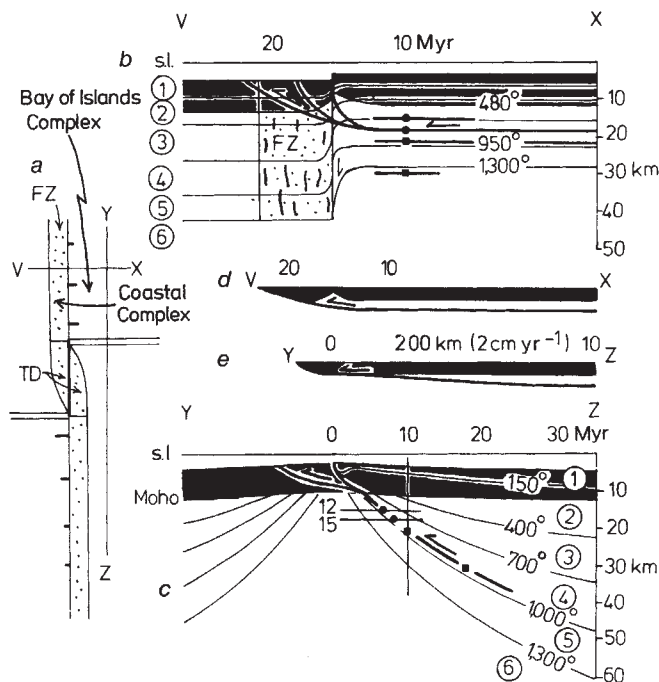


Fig. 4 Nucleation of obduction zone on a ridge transform plate boundary and its fracture zone extensions. *a*, Plan of ridge transform/fracture zone indicating lines of section in *b* and *d* (VX) and *c* and *e* (YZ). TD, transform domain; [■], polyphase-deformed rocks of transform/fracture zone. *b*, Section across a fracture zone with a contrast between 10 and 20 Myr lithosphere showing detachment of a 15-km-thick ophiolite sheet with a frontal portion of fracture zone rocks. FZ, fracture zone; black areas, mafic rocks; white areas, ultramafic rocks. 1, Brittle; 2, semi-brittle; 3, low-temperature ductile; 4, high-temperature ductile; 5, plastic; 6, low-velocity zone. [■], Range of pressure estimates from basal aureole³⁴; ●, bases of 12-km and 15-km-thick ophiolite sheets. *c*, Section across a ridge showing detachment of an ophiolite sheet along the 950 °C isotherm (solid ■ and ● as in *b*). *d*, True-scale section VX. *e*, True-scale section YZ generated at a half-spreading rate of 2 cm yr⁻¹.

inverted high-temperature metamorphic sole aureoles^{28,29}, initial detachment thicknesses of between 10 and 15 km²⁹, commonly carry primitive calc-alkaline arc volcanics, and may have oceanic fracture zone assemblages along the leading obduction edge^{18,30,31}. This is consistent with the detachment of a slice of oceanic crust and mantle, almost immediately after its generation, along a ductile zone at or near an accreting plate boundary crest or fracture zone, or a combination of both (Fig. 4). The earliest, highest temperature part of the obduction aureole in the Bay of Islands Complex was generated at ~950 °C (refs 32, 33) at the base of a 15-km ophiolite slab, a temperature/depth ratio appropriate for oceanic lithosphere <10 Myr old (Fig. 4c). Ophiolite obduction is easier shortly after generation because the 950 °C potential detachment surface becomes progressively deeper as the lithosphere ages (Fig. 4c). It is less clear what causes ridge → obduction zone conversion. Opening and closing of back and intra-arc basins has been suggested²⁸ but this is unlikely for Grampian ophiolites because they predate immediately volcanic arc development (Fig. 2f-j). Three principal mechanisms seem possible. First, changes in relative motion and intraplate and plate boundary stress patterns may result from regional or global plate boundary reorganization. Obduction by such a cause is likely to be roughly synchronous along great strike lengths of a continental margin, to predate arc development in the region and not to involve substantial syn-obduction rotations.

A second mechanism is related to an evolving trench/transform triple junction such as the Pacific/North American/Farallon junction on the early Tertiary Californian borderland. Flakes

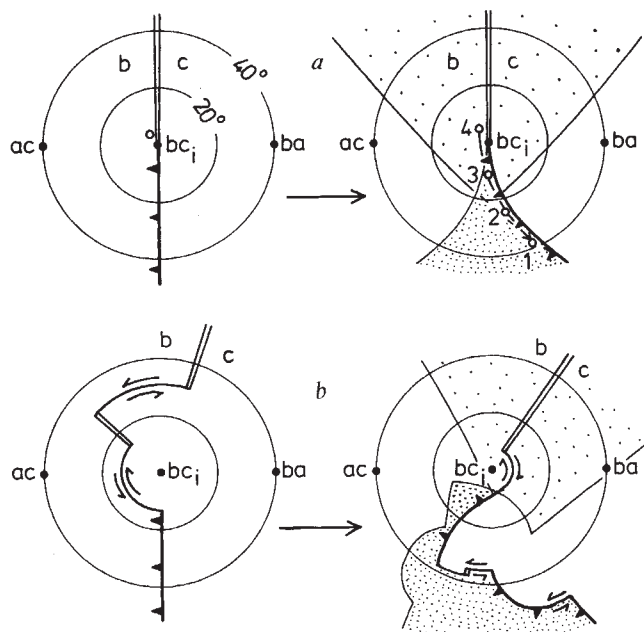


Fig. 5 Central portion of lower hemisphere equal-angle stereographic projections showing two plate boundary geometries that allow a close sequential relationship between ophiolite generation (double line) and beginning of obduction (barbed line). Plates *b* and *c* rotate around poles *ba* and *ac* respectively, which are fixed in the frame of reference of the projection and plate *a*. *bc*, The instantaneous rotation pole that describes the relative motion of plates *b* and *c*. [■], Area of plate *c* subducted beneath plate *b* after 60° of rotation of plates *b* and *c* around *ba* and *ac*, respectively; [■], area of plates *b* and *c* generated and not subducted, 1-4 represent localities in order of decreasing age at which ophiolite generation is shortly followed by obduction.

of newly-formed ophiolite could continuously transfer to a transform segment that had just been a subduction zone. This obduction mechanism would involve rather small flakes with substantial rotations and would terminate rather than precede volcanic arc activity. A third mechanism results from an inevitable consequence of plate boundary evolution—the progressive change in position of plate boundaries relative to the poles of rotation that describe their relative motion. Where ridge-fracture zone systems pass through or lie close to instantaneous rotation poles, a progressive change from plate divergence to convergence is possible³⁴ (Fig. 5a,b). This mechanism must generate diachronous change from spreading to obduction and is unlikely to cause substantial syn-obduction rotations.

The first and third mechanisms seem likely for large-scale ophiolite obduction, where ophiolite generation generally precedes arc growth. An example of the first mechanism may be the roughly synchronous Neotethyan late Cretaceous obduction from Cyprus to the Himalayas. The progressively later growth and demise of the Ordovician arc along-strike to the south-west within the Appalachian/Caladonian Orogen is best explained by the third mechanism.

Obduction detachment at a fracture zone^{18,30} produces a different configuration from detachment at a ridge crest^{28,29}. Fracture zone obduction (Fig. 4b,d) will involve an ophiolite nappe of approximately uniform across-strike but varying along-strike thickness, with a sheeted dike trend normal to the detachment line. Frontal nappe assemblages will consist of mafic/ultramafic assemblages generated along a fracture zone with complicated polyphase structural and magmatic histories, which form the principal obduction aureole protoliths. Assuming an obduction detachment surface dip of 5° and a converge rate of 2 cm yr⁻¹, the first gabbroic protoliths near the base of the oceanic crust will take 4 Myr to reach the highest aureole temperature and the uppermost oceanic crust will take 8 Myr.

By contrast, ridge crest obduction will generate an ophiolite nappe with approximately constant along-strike thickness, but thinning in the direction of obduction with a sheeted dike trend parallel with the detachment line. Frontal nappe assemblages will consist of newly generated oceanic crust and mantle and will form the earliest obduction aureole protoliths with temperatures as high as 1,200 °C at pressures as low as 2 kbar.

In Newfoundland, the evidence favours ophiolite detachment along a north-east-striking fracture zone (Coastal Complex), a continuation of the dextral transform portion of a long transform/short ridger system approximately parallel with, and a few hundred kilometres from, the early Ordovician continental margin³⁰. The obduction aureole comprises the first stages of a progressive northwestward and downward Arenigian nappe-stacking sequence that culminated with the emplacement of the whole Grampian nappe assemblage onto the platform during the mid-Ordovician. The progressive diminution of aureole temperature and pressure (Fig. 6f) indicates cooling and thinning of the ophiolite sheet during obduction. In a compressional regime, the thinning is unlikely to be tectonic in origin, nor can it have been due to syn-obduction erosion of a planar ophiolite sheet because the highest parts of the oceanic crust are preserved in the Bay of Islands Complex. Also, the earliest stages of thrusting of a 15-km sheet of 10 Myr ophiolite across a 20-Myr oceanic lithosphere would involve wholly submarine obduction with water depths of ~3 km. Even during the earliest stages of late D1/D2 progressive stacking of Dalradian continental margin sediments, shortening was occurring in a zone of thin continental crust and, therefore, emergence would have taken place only when the crust and sediments were restacked to above 30 km¹².

This event is witnessed by the Llanvirnian Crabb Brook³⁵ ophiolitic clastics (Fig. 2e) that rode on the Bay of Islands ophiolite complex. Thinning of the ophiolite nappe was probably mainly due to D3 buckling and erosional stripping, accounting for steeply dipping complete ophiolite sections transected by the final obduction thrust (Fig. 6d). During this early Arenigian early obduction phase, the obduction aureole and the highest Dalradian rocks in ophiolitic wildflysch sequences, such as those of Clew Bay³⁶, assume great significance because they probably record a direct relationship between structure and fabric and the obduction slip direction, which in turn is likely to be, more or less, the direction of relative motion of the plate. The aureole, in particular, will record the very earliest convergence slip direction following and perhaps progressively changing from the ridge transform direction, which will be preserved in the frontal portion of the ophiolite sheet and, more obscurely, in ophiolitic blocks in the highest wildflysch. Complicated structural sequences in mafic protoliths in metamorphic belts with younger structural sequences must be viewed, therefore, with extreme caution; they may represent obducted fracture zone rocks and not portions of an older crystalline basement. Systematic studies of early obduction structures have not been undertaken and are of paramount importance in attempts to relate structural geology to plate tectonics.

As D3 shortening progressed, the regional plane strain that had characterized D1 and D2 led to a progressive loss of the relationship between slip vector and fabric and to more complicated regional strains, partly driven by body forces involving flaking, wedging and rotation, with axial extensions and consequent strike-slip motions in a locally 70 km-thick crust. D3 shortening, with complexities probably partly due to the irregularities of the Laurentian rifted margin, choked the obduction zone, terminated the arc and led to D4 rotation²⁶ and retrocharriage and to northward subduction beneath, and uplift of, the Grampian tract (Fig. 6d,e). Mafic intrusions occur in several parts of the Grampian tract (Cashel Suite in Connemara, Newer Gabbros in Scotland) and predate D3. If they are the roots of arc volcanics³⁷, this model is invalidate, at least for the British Isles, because they would imply northward subduction during the Arenigian. They may, however, have been

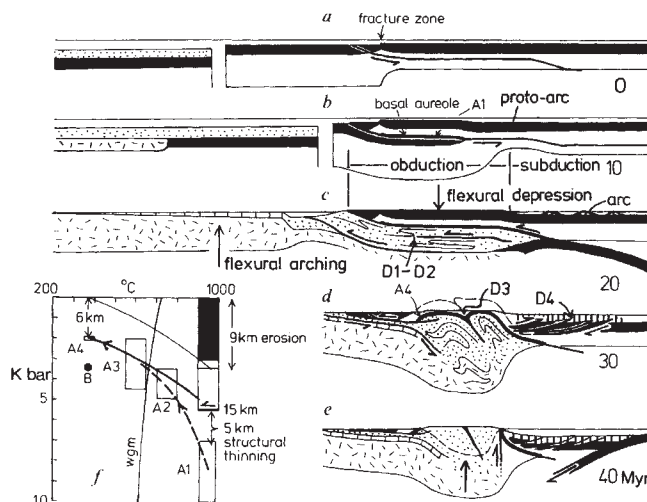


Fig. 6 a-e, Approximately true-scale schematic sections to account for the Grampian Orogeny, illustrating the progressive obduction of an ophiolite sheet across, and the sequential stacking of, a continental rise and continental shelf, the shortening and thickening of the continental margin rocks and the consequent flip in subduction polarity; for symbols see Fig. 3. f, Pressure-temperature plot of rocks of the basal aureole³². A1, two-pyroxene amphibolites; A2, amphibolites with trondhjemitic partial melts; A3, garnet-epidote amphibolites; A4, actinolite-greenschists; wgm, wet granite melting curve; B, crossite-bearing metamorphics.

developed in strike-slip pull-aparts or by delamination of the lithosphere³⁸ during shortening.

Displaced terranes and the closure of Iapetus

The Bay of Islands fracture zone model³⁰ implies a right-lateral component of Tremadocian/Arenigian relative plate motion across the Grampian Zone. Post-Llandeilian relative motion, however, until the late Silurian to mid-Devonian diachronous closure of Iapetus, involved a substantial component of left-lateral displacement. Very large sinistral late Devonian-Carboniferous displacements have been suggested^{39,40} to have occurred along the Appalachian/Caledonian chain, but the geological evidence clearly shows Carboniferous motion to have been right-lateral^{12,41}. We believe that all sinistral motion occurred between Caradocian and mid-Devonian times, based on the following evidence. The Great Glen/Leannan Fault and other roughly parallel faults moved during the early Devonian⁴² and probably have a total cumulative offset of a few hundred kilometres. Extensional bends in associated shear zones were critical in controlling the emplacement of granite plutons⁴³. The Highland Boundary Fault truncates the Dalradian terrane and juxtaposes it with the Midland Valley, which appears to consist of a Grenville basement stretched and underplated by a mafic lower crust during the latest Precambrian (B. J. Upton, personal communication) with superposed early Ordovician ophiolite obduction, a terrane very similar to the Long Range of southwest Newfoundland. Their original contiguity implies a sinistral displacement of at least 1,000 km which probably occurred from late Silurian to the end of the early Devonian, giving a slip rate of ~5 cm yr⁻¹. This large sinistral motion would account for the entrainment of a wide variety of Ordovician and Silurian rocks along the Highland Border⁴⁴. This further implies that the clastic source of the Southern Uplands accretionary prism, which must have travelled at least as far as the Midland Valley, cannot have been the Scottish Highlands, a notion supported by the fact that the Lower Devonian rocks of the Midland Valley have a source other than the Scottish Highlands⁴⁵.

In western Ireland, the Dalradian rocks of Connemara are in an anomalous position to the south of the Clew Bay Ophiolite Zone and the Ordovician volcanic rocks of the South Mayo Trough^{46,47}. The latter cannot have been thrust across the

Dalradian because their very preservation means that they escaped the large uplifts and denudations (>15 km) suffered by the Grampian metamorphics and hence cannot be underlain by Dalradian rocks. Upper Llandoveryan strata rest unconformably across a concealed contact between the Ordovician rocks of the South Mayo Trough and the Connemara Dalradian where the Lough Nafooy Fault Zone was a major controlling influence on Arenigian volcanism and sedimentation⁴⁸. This fault zone is interpreted here as a major post-Llandeilian, pre-Upper Llandoveryan strike-slip fault along which Connemara was displaced from the set by ~500 km. Lastly, early Devonian terminal closure of Iapetus in north-central Newfoundland had a strong sinistral component as evidenced by sets of sinistral Riedel faults in New World Island between the Lukes Arm and Reach Faults. The sum of all these post-Grampian sinistral motions is at least 1,800 km and had the effect of severely modifying, by strike-slip omission and repetition, the Grampian tract and its post-Llandeilian subduction-accretion neighbouring terrane to the south, and may have been responsible also for substantial pre-collisional modification to the southern margin of Iapetus such as the truncation of the Lake District forearc region⁴⁹. Perhaps the northern margin of Iapetus between Llandeilian and late Silurian times was similar to the Mesozoic-Cenozoic western margin of North America where complex plate interactions were driven by roughly strike/slip motion between the large plates.

Conclusions

The pre-Grampian margin of the Laurentian Shield in the Appalachians was a continental shelf-rise generated by latest Precambrian continental separation. Continental separation was heralded by the eruption of the Tayvallich and Lighthouse Cove mafic lavas. The Dalradian rocks were evidently laid down in a basin bounded to the south by a horst³. The pre-Grampian right-lateral phase of offshore ridge/transform tectonics may have been responsible for the removal of this crystalline horst (perhaps now the basement of the Ammonoosuc Zone in western New England), much as the opening of the Gulf of California is transporting a narrow Baja continental sliver northwards to open up the transform continental borderland of Mexico. The offshore ridge/transform boundary was converted, almost immediately and probably diachronously, along the length of the Appalachian/Caledonian Orogen, into an obduction zone during Tremadocian-early Arenigian times. It was the progressive northward obduction of a 15-km ophiolite sheet that stacked a subjacent Dalradian nappe pile, to the south of which grew an Arenigian volcanic arc on a Tremadocian ophiolite foundation. Grampian crustal shortening was below sea level for a substantial part of the early Ordovician until rapid D3 shortening and crustal thickening led to a flip in subduction polarity, the beginning of southward-verging subduction accretion and rapid uplift and stripping of the Grampian tract. The ophiolite nappe is preserved in the steep suture zone between the arc and the Dalradian terrane and where it reached the platform. In the uplifted and denuded axial part of the Grampian Zone, it is preserved only in high-level slide zones in lower grade regions.

The obduction model proposed here may be a template for the evolution of most Barrovian mountain belts of Grampian type that evolve prior to terminal oceanic closure. Major ophiolite obduction seems to occur long before continental collision and emphasizes the observation of Hess⁵⁰ that the great Alpine-type ultramafic belts were generated during the first major deformational events in the mountain belt that contains them. This massive scale of obduction appears to result from ridge-fracture zone → obduction zone conversion and may be significant with regard to global plate tectonics. As in the early Ordovician, a similar period of widespread obduction occurred in mid- and late Cretaceous times and was also temporally coincident with blueschists, arc activity, seamount-continent collision, continental margin orogeny and widespread platform carbonate signalling a high sea level and rapid subduction¹².

The post-Grampian sinistral motion of almost 1,800 km has

implications for the relationship between the Grampian margin of the Laurentian Shield, the Avalonian terrane and the Baltic Shield (Fig. 1). If this relative motion were carried along-strike between Greenland and Scandinavia, it would imply that the south-east-verging Scandinavian Caledonides were opposite Newfoundland and Ireland in immediately post-Grampian times. As a more plausible explanation, we suggest that a major oceanic triple-junction complex existed in the North Sea⁵¹ between the Scandinavian, British and North German Caledonides and that subduction along the latter absorbed a large part of the Avalonian-Laurentian relative motion. Considerable differences exist between the Grampian tract in the Appalachians and Caledonides. In the Appalachians, a narrow zone of Grampian thrusting and ophiolite obduction was completed by mid-Ordovician times and the Connemara Dalradian was stripped by late Llandoveryan times. In Scotland, the Grampian Zone is wider and thrusting continued into the Silurian in the Moine terrane⁴². A possible explanation is that a D4 subduction polarity change following D1-D3 obduction was followed shortly by Silurian continental collision between the Laurentian and Baltic Shields and by progressive oceanic closure, with a strong sinistral component, culminating in late Silurian to mid-Devonian collision between Avalonia and a semi-consolidated Laurentian/Baltic continent. This collision induced Devonian sinistral strike-slip between the Laurentian and Baltic Shields (Great Glen phase). Before terminal closure of Iapetus, major strike-slip faulting occurred along the Grampian borderland of Laurentia. Of the 1,800 km of Avalonia-Laurentia relative motion, about 500 km was absorbed by late Silurian-early Devonian shortening in the North German/Polish Caledonides, and the remainder accounted for the Devonian left-lateral displacement of Scandinavia relative to Laurentia.

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Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence

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Cloning and sequence analysis of cDNA for the Electrophorus electricus electroplax sodium channel indicate that this protein, consisting of 1,820 amino acid residues, exhibits four repeated homology units, which are presumably oriented in a pseudosymmetric fashion across the membrane. Each homology unit contains a unique segment with clustered positively charged residues, which may be involved in the gating structure, possibly in conjunction with negatively charged residues clustered elsewhere.

THE sodium channel is a membrane protein that mediates the voltage-dependent modulation of the sodium ion permeability of electrically excitable membranes^{1,2}. The ionic basis for the generation of action potentials has been well understood³. The initial increase and subsequent decrease in sodium ion permeability during an action potential implies that the sodium channel can exhibit at least three distinct functional states: resting (closed), activated (open) and inactivated (closed). A variety of pharmacological agents, including neurotoxins, affect the complex function of the sodium channel⁴. The use of binding assays based on the high specificity of such neurotoxins has permitted purification of the sodium channel from the electric organ of the eel *Electrophorus electricus*⁵⁻⁷ as well as from rat brain⁸, rat skeletal muscle⁹ and chick cardiac muscle¹⁰. It has been reported that the *Electrophorus* sodium channel⁷ and its avian counterpart¹⁰ consist of a single polypeptide of molecular weights 260,000-300,000 and 230,000-270,000, respectively, whereas its mammalian counterparts^{8,9} contain, in addition to the corresponding large polypeptide, two or three smaller polypeptides of molecular weights 37,000-45,000. The purified eel and rat sodium channel preparations have been shown to be functional when reconstituted into phospholipid vesicles¹¹⁻¹⁴.

To understand the molecular mechanisms underlying the selective ion transport and voltage-dependent gating operated by the sodium channel, it is essential to elucidate the primary structure of the constituent protein. Using recombinant DNA techniques, we have now cloned DNA sequences complementary to the messenger RNA coding for the sodium channel protein of *El. electricus* electroplax. Nucleotide sequence analysis of the cloned cDNA has revealed the complete amino acid sequence of this polypeptide.

Partial amino acid sequence

The initial approach used to clone cDNA sequences for the sodium channel protein was to combine immunological screening of a cDNA expression library with identification of the

isolated clones by comparison with the partial amino acid sequences determined by peptide analysis. The sodium channel protein from *El. electricus* electroplax was solubilized with Lubrol-PX and purified by chromatography on DEAE-Sephadex and gel filtration on Sepharose 6B (refs 5, 6), followed by gel filtration on Sepharose 4B in denaturing conditions⁷ and gel permeation HPLC (see Fig. 1 legend). This procedure yielded a homogeneous preparation (Fig. 1A). The purified protein was digested with trypsin, and the resulting peptides were fractionated by reverse-phase HPLC (Fig. 1B). After rechromatography (Fig. 1C), five peptide fractions (fractions Ib, IIa, IIIb, IVa and Vb) were isolated and analysed for amino acid sequence with the use of a gas-phase sequencer¹⁵. Six peptide sequences were thus determined (Fig. 1D); fraction Ib proved to contain two peptides. Attempts to determine the amino-terminal sequence of the whole sodium channel protein by Edman degradation were unsuccessful.

Isolation of cDNA clones

A library of cDNA clones was constructed with poly(A)-containing RNA from *El. electricus* electroplax as template and the plasmid expression vector pUC8 (refs 16, 17). Screening of the cDNA library¹⁷ with rabbit antiserum to the purified *El. electricus* sodium channel protein yielded four immunologically positive clones from $\sim 8 \times 10^4$ transformants. Plasmid DNA was isolated from the four clones, and the cDNA insert of each clone was subjected to blot hybridization analysis¹⁸ with a synthetic oligodeoxyribonucleotide probe containing all possible cDNA sequences predicted from the partial amino acid sequence Thr-Phe-Phe-Glu-Pro of the tryptic peptide Vb (see Fig. 1D, e); the third position of the proline codon was not included. Only one of the four clones, pSCH1 (see Fig. 2), carried a hybridization-positive cDNA insert. Nucleotide sequence analysis¹⁹ of this cDNA insert revealed that clone pSCH1 contained a 426-base pair (bp) cDNA insert including the coding sequence for the whole known amino acid sequence

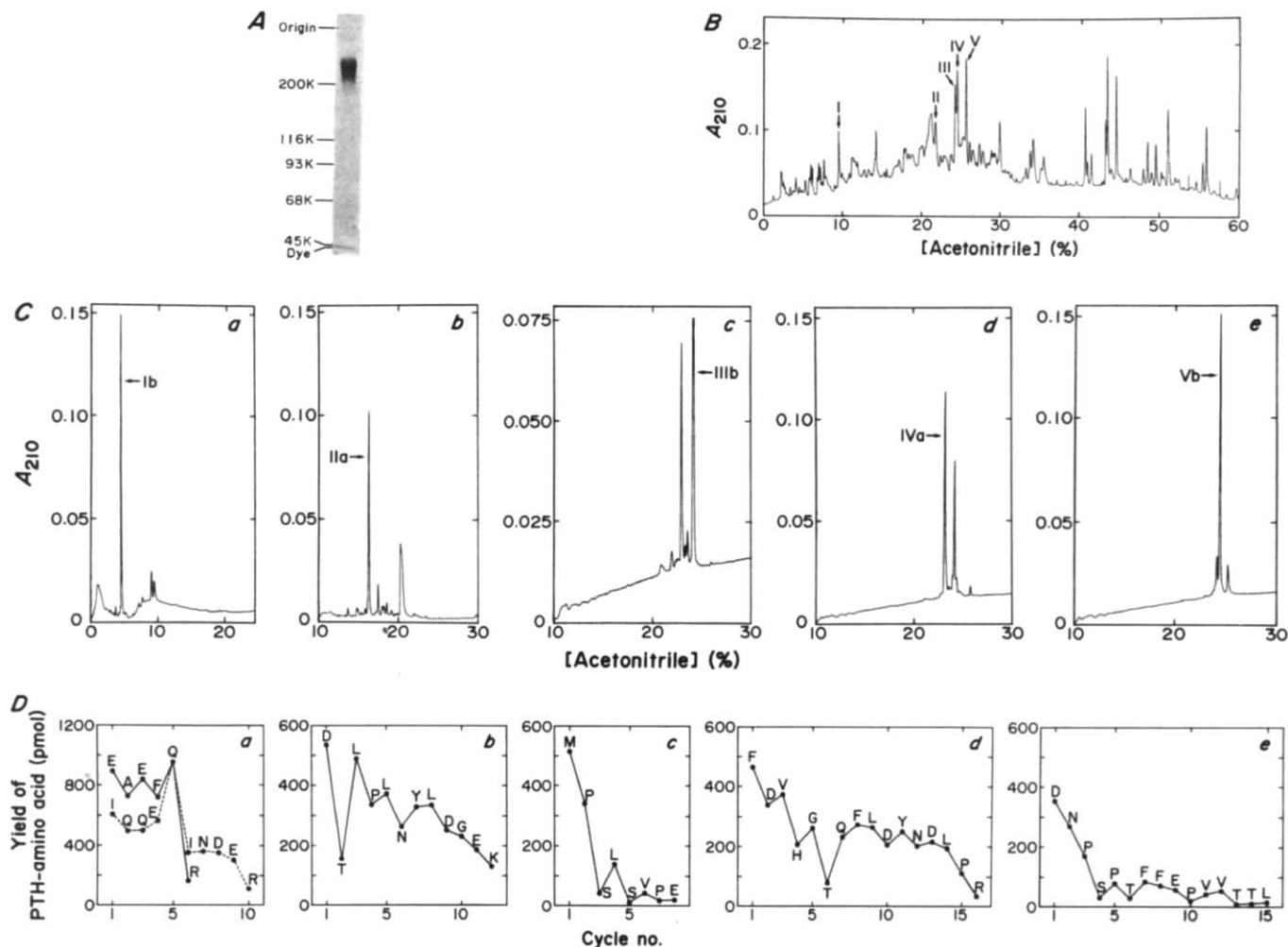


Fig. 1 Purification and partial amino acid sequence analysis of the *El. electricus* sodium channel protein. **A**, Electrophoretic analysis of the purified protein on an SDS-6% polyacrylamide gel¹⁵. The size markers used were chicken ovalbumin [molecular weight (M_r) 45,000 (45K)], bovine serum albumin (M_r 68K), rabbit muscle phosphorylase *b* (M_r 93K), *Escherichia coli* β -galactosidase (M_r 116K) and rabbit myosin heavy chain (M_r 200K); bromophenol blue served as tracking dye. The polypeptides were stained with Coomassie brilliant blue. **B**, Reverse-phase HPLC of tryptic peptides. The tryptic digest was directly loaded onto a Chemcosorb 3μ C₁₈-H column (8 $\times$ 75 mm; Chemco) equilibrated with 0.1% trifluoroacetic acid, and a linear gradient of 0–60% acetonitrile was applied over 80 min at a flow rate of 2 ml min⁻¹; a Hitachi 635 HPLC system with a solvent programmer was used. The eluate absorbance at 210 nm was recorded with a Hitachi 41 UV monitor. Five fractions (I–V) corresponding to absorbance peaks were collected. **C**, Second reverse-phase HPLC. Fractions I, II, III, IV and V (**a**, **b**, **c**, **d** and **e**, respectively) were further purified by reverse-phase HPLC on a Novapak 5μ C₁₈ column (3.9 $\times$ 150 mm; Waters). The column was equilibrated with 0.1% ammonium acetate buffer, pH 6.0 (**a**), 0.1% ammonium acetate buffer, pH 6.0, containing 10% acetonitrile (**b**) or 10 mM ammonium formate buffer, pH 4.0, containing 10% acetonitrile (**c**–**e**), and a linear gradient of 0–60% (**a**), 10–60% (**b**) or 10–50% acetonitrile (**c**–**e**) over 60 min was used at a flow rate of 1 ml min⁻¹. Peak fractions (Ib, IIa, IIIb, IVa and Vb) were collected. **D**, Sequence analysis of peptides. Fractions Ib (**a**), IIa (**b**), IIIb (**c**), IVa (**d**) and Vb (**e**) were subjected to amino acid sequence analysis with a gas-phase sequencer¹⁵ (model 470A, Applied Biosystems). Phenylthiohydantoin (PTH)-amino acids were analysed by HPLC at 50 °C using an Ultrasphere ODS column (4.6 $\times$ 250 mm; Altex) with a three-solvent gradient (acetonitrile, water and 40 mM sodium acetate buffer, pH 5.6) at a flow rate of 1 ml min⁻¹. The yield of PTH-amino acids at each cycle of Edman degradation is shown; the one-letter amino acid notation is used. Fraction Ib was a mixture of two peptides, whose sequences were assigned as indicated, assuming that glutamine is shared by the two peptides at the fifth amino acid position.

Methods: Approximately 2 mg of the partially purified sodium channel protein (Sephacrose 6B fraction^{5,6}), derived from about 200 g of *El. electricus* electric organ (World Wide Scientific Animals), was further purified by gel filtration on Sepharose 4B in denaturing conditions⁷. Fractions enriched in sodium channel protein (derived from ~600 g of electric organ) were combined and alkylated at 25 °C for 30 min with 0.1 M iodoacetamide in the presence of 0.13 M 2-mercaptoethanol, 0.1% SDS and 0.4 M Tris-HCl buffer, pH 8.0. After desalting with a Biogel P-100 column (1.5 $\times$ 20 cm) equilibrated with 0.02% SDS, the sodium channel protein was finally purified by gel permeation HPLC on a TSK 4000 column (7 $\times$ 600 mm; Toyo Soda) using 50 mM sodium phosphate buffer, pH 7.5, containing 0.1% SDS at a flow rate of 0.5 ml min⁻¹; the yield was ~300 μ g. The purified material was dialysed against water, lyophilized and dissolved in 90 μ l of 0.1 M ammonium bicarbonate containing 10 mM CaCl₂. TPCK-treated trypsin (Worthington) was added at a sodium channel protein/trypsin ratio of 100. After incubation at 37 °C for 12 h, a further identical aliquot of TPCK-treated trypsin was added, and the incubation was continued for additional 12 h.

of the tryptic peptide Vb. When total RNA from *El. electricus* electroplax was subjected to blot hybridization analysis²⁰ with the cDNA insert of each of the four clones as probe, an RNA species of ~8,000 nucleotides was hybridizable not only with clone pSCH1 but also with another clone, pSCH4 (see Figs 2, 4); no hybridizable RNA was detected with the two remaining clones. This suggested that both pSCH1 and pSCH4 carried a cDNA sequence for the sodium channel protein.

To clone longer cDNA sequences, we next constructed a cDNA library, using poly(A)⁺ RNA from *El. electricus* electroplax and the plasmid vector elaborated by Okayama and Berg²¹. Screening of this library²² by hybridization with the combined cDNA inserts of clones pSCH1 and pSCH4 yielded 14 hybridization-positive clones from ~2 $\times$ 10⁵ transformants. All 14 clones

hybridized with the pSCH1 probe, whereas only 6 of them hybridized with the pSCH4 probe. Clone pSCH14, which apparently harboured the largest cDNA insert among the 14 clones, contained the whole cDNA sequence carried by pSCH1, but did not extend to the 5' end of the cDNA carried by pSCH4 (see Fig. 2). Therefore, additional ~1 $\times$ 10⁶ transformants from the same Okayama-Berg library were screened with a hybridization probe derived from the 5'-terminal portion of the cDNA insert of pSCH4. Thus, five hybridization-positive clones were isolated. One of these clones, pSCH50, which apparently contained the largest cDNA insert among them, extended beyond the 5' end of the cDNA insert of pSCH4 (see Fig. 2).

Because clone pSCH50 apparently did not carry the entire protein-coding sequence, however, additional clones harbouring

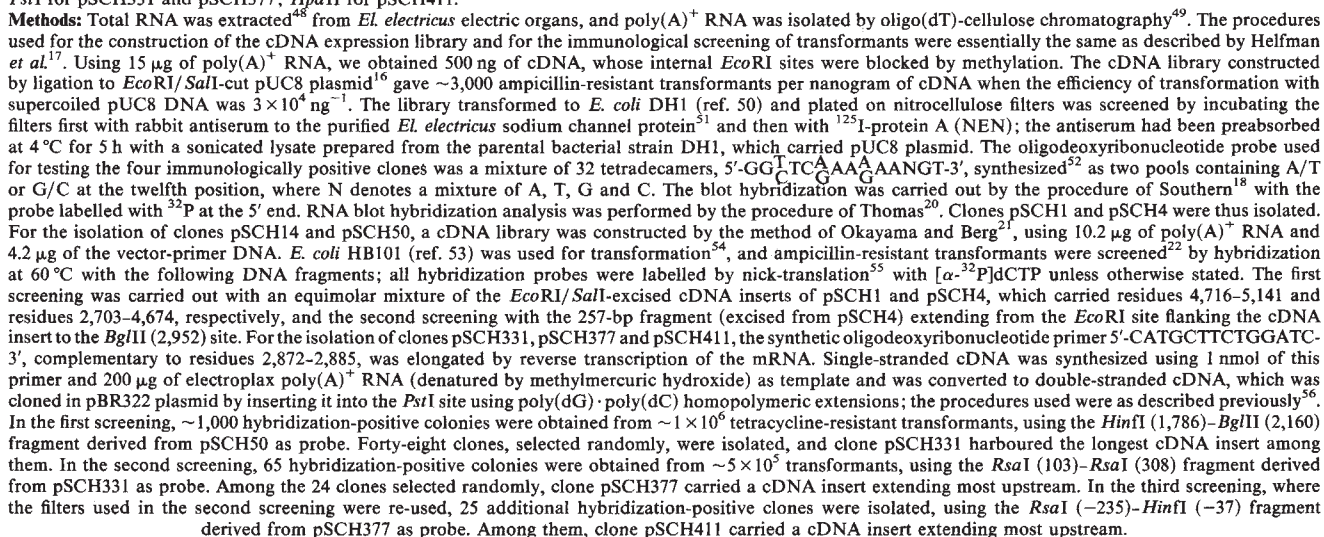


Figure 3 shows the 7,230-nucleotide sequence (excluding the poly (dA) tract) of the cDNA encoding the *El. electricus* sodium channel protein, determined using clones pSCH1, pSCH4, pSCH14, pSCH50, pSCH331, pSCH377 and pSCH411. No nucleotide difference was observed among the cDNA sequences determined with the individual clones, except that the polyadenylation site differed by four nucleotides between clones pSCH14 and pSCH50 (see below). All of the six partial amino acid sequences determined by peptide analysis (see Fig. 1D) were found to be encoded by the cDNA sequence in the same reading frame (amino acid residues 420–425, 432–441, 861–872, 1,596–1,611, 1,677–1,691 and 1,774–1,781). By using this reading

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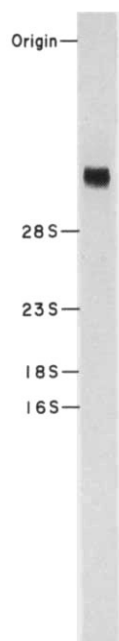


Fig. 4 Autoradiogram of blot hybridization analysis of *El. electricus* electroplax RNA with a cDNA probe. Total RNA (20 µg) was analysed by the procedure of Thomas²⁰. The hybridization probe used was the *Ava*II (193)–*Ava*II (1,675) fragment derived from clone pSCH411. The size markers used were calf and *E. coli* rRNA³⁷. Blot hybridization with other probes derived from different regions of the cloned cDNA exhibited a single hybridization-positive band with the same size; the probes used were the 577-bp fragment (excised from clone pSCH411) extending from the *Ava*II site flanking the cDNA insert to the *Ava*II (193) site, the *Ava*II (1,861)–*Hinc*II (2,422) fragment derived from clone pSCH411, the *Eco*RI/*Sal*I-excised cDNA insert of clone pSCH4 (carrying residues 2,703–4,674) and that of clone pSCH1 (carrying residues 4,716–5,141).

frame, the amino acid sequence of the cryptic portion of the sodium channel protein was deduced (Fig. 3). The translational initiation site was assigned to the methionine codon composed of nucleotide residues 1–3 because this is the first ATG triplet that appears downstream of the nonsense codon TAG (residues –42 to –40) found in-frame. This assignment is supported by the fact that the sequence surrounding the ATG triplet agrees with the favoured sequence that flanks functional initiation codons in eukaryotic mRNAs²³, that is, $\hat{A}XXAUGG$, where X can be any nucleotide; the sequences flanking the following 13 methionine codons do not match this favoured sequence. Reverse transcriptase-mediated elongation of the *Hind*III (–250)–*Hinc*II (–307) fragment derived from the 5'-terminal region of clone pSCH411 yielded a cDNA transcript of ~600 nucleotides, suggesting that no more than ~500 nucleotides are missing from the cDNA we have cloned. This is consistent with the size of the mRNA coding for the sodium channel protein (~8,000 nucleotides) as estimated by blot hybridization analysis²⁰ of electroplax RNA (Fig. 4). The codon specifying the valine residue at position 1,820 is followed by the translational termination codon TAA. The length of the 3'-non-coding region of the cDNA is 1,386 nucleotides (clone pSCH14) or 1,382 nucleotides (clone pSCH50), excluding the poly(dA) tract. Two overlapping copies of the polyadenylation signal²⁴ AATAAA (residues 6,831–6,836 and 6,827–6,832) are found 16 nucleotides upstream from the poly(dA) tract of the respective clones.

From the cDNA sequence, we conclude that the *El. electricus* sodium channel protein is composed of 1,820 amino acid residues. The molecular weight of the protein is calculated to be 208,321. This value agrees with the reported molecular weight of the protein moiety of the sodium channel protein, whose carbohydrate content is ~29% by weight⁷. The amino acid composition of the sodium channel protein predicted from the cDNA sequence compares reasonably well with that determined⁷. There are 10 potential sites of *N*-glycosylation²⁵, that is, Asn-X-Ser/Thr, where X can be any amino acid except proline²⁶ (asparagine residues at positions 205, 278, 288, 317, 591, 690, 797, 1,160, 1,174 and 1,806); six of them are assigned to the extracellular side of the membrane (see below).

The protein structure

Homology matrix comparison²⁷ of the amino acid sequence of the sodium channel protein reveals the presence of four internal repeats that exhibit sequence homology. Figure 5 shows the alignment of the amino acid sequences of the four repeats, which consist of residues 111–419 including a non-homologous seg-

ment of residues 285–342 (repeat I), residues 555–807 (repeat II), residues 989–1,281 including a non-homologous segment of residues 1,172–1,194 (repeat III) and residues 1,311–1,587 including a non-homologous segment of residues 1,490–1,505 (repeat IV). Of the positions aligned, 54%, 54%, 43%, 50%, 54% and 47% are occupied by identical residues or residues considered to be favoured substitutions for the I/II, I/III, I/IV, II/III, II/IV and III/IV pairs, respectively; the non-homologous segments have not been included in the calculation, and gaps have been counted as one substitution regardless of their length. Statistical tests²⁷ show that the sequence similarity among the four repeats is highly significant, except for the I/IV pair, the probability of occurrence by chance being less than 10^{-9} for the I/II, I/III, II/III and II/IV pairs and 1.3×10^{-3} for the III/IV pair. This observation strongly suggests that the four repeated homology units of the sodium channel protein have evolved from a single common ancestor by internal duplications.

The amino acid sequence of the sodium channel protein was analysed for predicted secondary structure²⁸ and for local hydrophathy²⁹ (Fig. 6A). The segments that can form structures of α -helix or β -sheet extend over a wide range of the protein molecule. The hydrophathy profile along the polypeptide chain indicates that each repetitive homology unit contains five hydrophobic segments (S1, S2, S3, S5 and S6) at equivalent positions. In addition, each unit contains a characteristic segment with strong positive charge (S4) located between segments S3 and S5.

Segments S5 and S6 in each repeat correspond to highly hydrophobic regions with predicted secondary structure that comprise a continuous stretch of 24–38 uncharged amino acid residues including many nonpolar residues. These hydrophobic regions are flanked on both sides by charged residues. Because such a sequence is characteristic of transmembrane protein segments, segments S5 and S6 most probably traverse the membrane, forming α -helical structures^{30–32}.

Segments S1, S2 and S3 in each repeat represent hydrophobic regions with predicted secondary structure consisting of 18–28 amino acid residues that are largely nonpolar but include a few charged residues. Negatively charged residues predominate in segments S1 and S3, whereas both positively charged and negatively charged residues are present in segment S2, so that this segment generally has no net charge. If an α -helical structure is assumed for these segments, the charged side chains in them are clustered largely on one side of the helix, the opposite side being occupied mostly by nonpolar side chains. Thus, segments S1, S2 and S3 can also span the membrane, forming amphipathic α -helical structures.

Segment S4 in each repeat represents a positively charged region with predicted secondary structure comprising 21 amino acid residues including five to seven arginine or lysine residues; arginine residues predominate. This segment exhibits a unique structural feature in that the arginine or lysine residues are located at every third position. The residues intervening between these basic residues are mostly nonpolar. If an α -helical structure is assumed for this segment, the positively charged side chains would be distributed along a spiral line, making a three-quarter to one turn. Alternatively, if a 3_{10} -helical structure is assumed, they would lie on one side of the helix so that the segment would be strongly amphipathic. On the other hand, if a β -sheet structure is assumed, they would extend alternately towards both sides of the peptide backbone.

It seems reasonable to postulate that the four repeated homology units are oriented in a pseudosymmetric fashion across the membrane. If this is the case, each unit should contain an even number of transmembrane segments because no additional hydrophobic segments are predicted outside the homology units (see Fig. 6A). The transmembrane segments are most likely to be involved directly or indirectly in the formation of the ionic channel. As the minimum cross-section of the channel is considered to be $3 \times 5 \text{ \AA}$ (refs 33, 34), at least one helical segment from each repetitive unit would take part in constructing the inner wall of the ionic channel at the narrowest point. The remaining helical segments may surround those forming the

Fig. 5 Alignment of the amino acid sequences of the four internal homology units. The sequences of repeats I (top), II (second row), III (third row) and IV (bottom) are aligned. The one-letter amino acid notation is used, and the numbers of the residues on each line are given on the right side. Identities or favoured amino acid substitutions among three or four residues at one aligned position are indicated by boxes. Favoured amino acid substitutions are defined as pairs of residues belonging to one of the following groups: S, T, P, A and G; N, D, E and Q; H, R and K; M, I, L and V; F, Y and W (ref. 58). Gaps (-) have been inserted to achieve maximum homology. The non-homologous segments in repeats I, III and IV are shown by lines; the sum of residues present in each non-homologous segment is given in parentheses. Segments S1-S6 are indicated.

I	IRRGAI-RVFNNSAFNFFN--FTTFSNCIFMTISNPPAWK----	IVVEYTL--FTGTITFEVIVKVLGRFCIGHFTFLDPWNLDF	111-183
II	LKKVWH-FVMDPFTDLFI--LCIHLNLFMSIEHHPNES--FQSLUSAGNLVFTTIFAAEMVLKIIA-LDPYYFQ--QTWNTFDS	555-635	
III	LRRITCYTIVHEDYFETFI--FMILLSSGVLAPEDIYWRRAVIVKILEYADKVFIVVFVEMLLKWAYGFRKYFT--DAWCWLDFF	989-1071	
IV	VGGVVDIV-TQPTDIFMALICINMVA--MVESEDSQGV--KKDILISQTNVILFVILFTECLLKL--LRGYFT--VGMNVDFDF	1311-1390	
	S1	S2	S3
I	SNVTMTITIT-----EFIDL-----RNVSALRTFRVLRAKLTITITFPGLKTIIVRALITEPMKQMGDGVVITLTVFSDAVFTLAGMGL	190-262	
II	IVLSLLE-----GLSNM-----GGMSVLSRLRLRIEKLAKSWPTLNTIKITCNSVGALGNLITVLATIVFIFALVGLQL	636-709	
III	VIVGASIMGITSSLLGYEEL-----GATKILRTIRALRPLRALSRFEKMGKVVVRAALLGAIIPSNVLLVLCMFNLIIFSIMGVNL	1072-1150	
IV	AVVVISIIG-----LILLSDITKCYVSPTLFRVIRLARTARVLRIRAKGIRITLFL--ALMMSLPALFNJGLLFLFIFISIFGMSN	1391-1472	
	S4	S5	
I	FMGNLRH-KCI RWPISNV-IT-DYE-----NVDNFAWTFCLFLRLML-QDYVENVLY--QMTLRAAGKSY-MVFFIT--	263-383	
II	FGKNYKEYVIC--KISDCELPKMH-----MNDFFH-SFLIVFRALC-GEWETFMW--DCMEVGSGVPMCLAVYM--	710-771	
III	FAG--KFYRCI--NTITDDEILPVEE-----NVDNAGNGVLSLLQVSTFKGMDIYAAVDSREVEDQRIYEINVYVYL	1151-1242	
IV	FAYVKKD-----GGVDID-IF-NFE-----GNDGLLHLLNTGPPDC-DPDVEN--PGTIVRGNCNPGKGLITFEC--	1473-1548	
	S6		
I	--PVITGLSFYILNLLAVVAMAYEEGQATILA--EAEQEK	384-419	
II	--MVTIGNLVNLFLALLLSFSSDNLISIE--EDDEIV	772-807	
III	YFVIFIVGAFFTNLFGVITDNFNRRQKGLGG--EDLFM	1243-1281	
IV	--SYIILSFLVVMYIAITIEFGVAGKEISDLCEDDFV	1549-1587	
	S6		

channel, or some of them may face the channel in its dilated portion. In view of the strong hydrophobicity and the uncharged nature of segments S5 and S6, there seems to be little doubt that they represent transmembrane segments. It is likely that two of the three segments S1, S2 and S3 also traverse the membrane. The negatively charged side chains in segment S1 and/or segment S3 may be related to the ion selectivity of the sodium channel³⁵. The presence of a continuous stretch of 31 uncharged residues in the S1 region of homologous repeat I seems to favour the view that segment S1, together with segment S2 (or S3), spans the membrane.

The sodium channel protein, in contrast to many other transmembrane proteins³⁶, does not possess an amino-terminal sequence characteristic of the signal peptide³⁷. There are a number of such precedents³⁸⁻⁴¹, and transmembrane proteins devoid of a cleavable signal peptide are considered to have their amino terminus on the cytoplasmic side of the membrane³¹, as this is in fact the case for the erythrocyte anion transport protein band III (ref. 38). On the basis of the considerations mentioned above, the transmembrane topology of the sodium channel protein molecule has tentatively been assigned as shown in Fig. 6B. The possibility that segment S3, instead of segment S2 (or S1), traverses the membrane or that all six segments S1-S6 span the membrane cannot be excluded. In view of the ion selectivity of the sodium channel³⁵, however, it seems rather unlikely that segment S4, which has strong positive charge, is involved in the channel lining.

The voltage-dependent operation of the sodium channel implies that the structure responsible for gating is charged and is able to move in the membrane field, as postulated by Hodgkin and Huxley³ and subsequently confirmed by measurement of

the gating current⁴². The gating current is outward during activation, and available evidence suggests that both the activation and inactivation gates are located near the inner membrane surface⁴³. Noteworthy in this context is the presence of the unique, positively charged segment S4 in each of the four repeated homology units (see above) as well as the presence of a large excess of negatively charged amino acid residues over positively charged residues in the region between segment S6 of homologous repeat II and segment S1 of the repeat III. There are four segments each having 5-12 clustered acidic residues (segments composed of residues 802-806, 847-857, 894-910 and 942-955) in this region, which is assigned to the cytoplasmic side of the membrane (see Fig. 6B). These acidic segments are located at regular intervals of 31-40 residues, corresponding to the four hydrophilic segments observed in the hydropathy profile (see Fig. 6A). It is attractive to hypothesize that each of the four positively charged segments, possibly in conjunction with each of the four negatively charged segments, acts as voltage sensor, thus being involved in an activation gate. The presence of four homologous structures in a single sodium channel molecule would be consistent with the sigmoid activation kinetics characteristic of this channel³. Armstrong⁴⁴ postulates the presence of four to six closed states rather than three³ and claims that the successive transitions among them are fast and are each associated with a charge movement that amounts to only half that in the transition from the last closed state to the open state. Of interest in this context is the finding that the charge carried by the four individual acidic segments varies about twofold. Concerted movement of many negative groups and/or many positive groups as clusters even by a small distance could account for the gating current⁴⁴ and would induce a

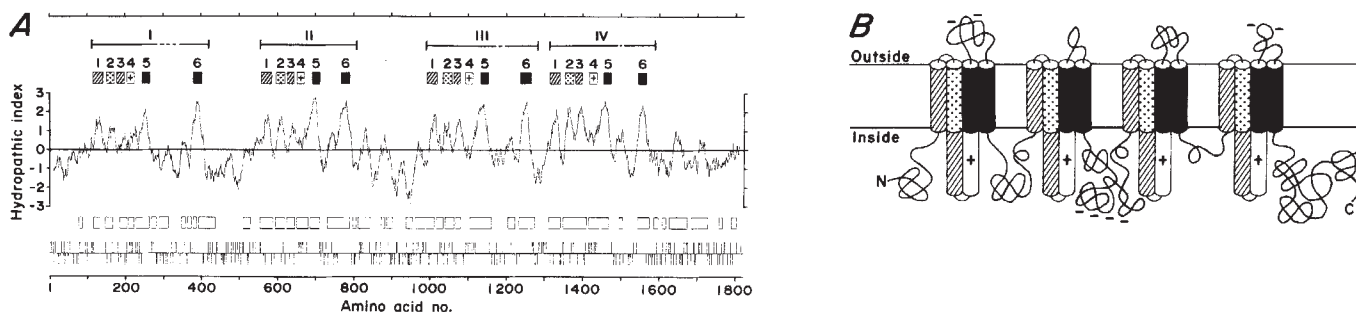


Fig. 6 Hydropathy profile and predicted secondary structures (A) and proposed transmembrane topology (B) of the sodium channel protein. In A, the averaged hydropathic index of a nonadecapeptide composed of amino acid residues $i-9$ to $i+9$ has been plotted against i , where i represents amino acid number; the hydropathy indices of individual amino acids have been taken from ref. 29. The positions of positively charged residues (lysine and arginine) and negatively charged residues (aspartic acid and glutamic acid) are indicated by upward and downward vertical lines, respectively. The locations of homologous repeats I, II, III and IV are shown by bars, and those of the non-homologous segments within the repeats by dashed lines. The positions of the characteristic segments S1-S6 in each repeat are indicated as follows: hatched boxes, segments S1 and S3 which generally contain negatively charged residues; stippled boxes, segment S2 with both positively charged and negatively charged residues; boxes with a plus sign, segment S4 with regularly spaced, positively charged residues; closed boxes, segments S5 and S6 without charged residues. In B, the four homology units spanning the membrane, which presumably surround the ionic channel, are displayed linearly. Segments S1-S6 in each unit are shown by cylinders marked as in A, and segments S1, S2, S5 and S6 are located within the membrane. Alternative possibilities of the transmembrane topology are discussed in the text. The regions containing clustered negatively charged residues are indicated by minus signs.

conformational change of the protein to open the channel. It is also intriguing to speculate that the region encompassing the negatively charged segments, after such a conformational change upon depolarization, might in turn be involved in the inactivation of the channel, under the assumption that the activation and inactivation mechanisms are coupled⁴⁴.

The region between segments S5 and S6 of homologous repeat I as well as IV is also rich in acidic residues, which are not as clustered as those in the negatively charged region discussed above. As the region between segments S5 and S6 is assigned to the extracellular side of the membrane, it is tempting to

hypothesize that the negatively charged residues in this region of homologous repeats I and IV, facing the outer end of the channel, are also involved in the ion selectivity of the channel and that these areas contain the binding sites for basic neurotoxins⁴.

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The reactivity of anti-peptide antibodies is a function of the atomic mobility of sites in a protein

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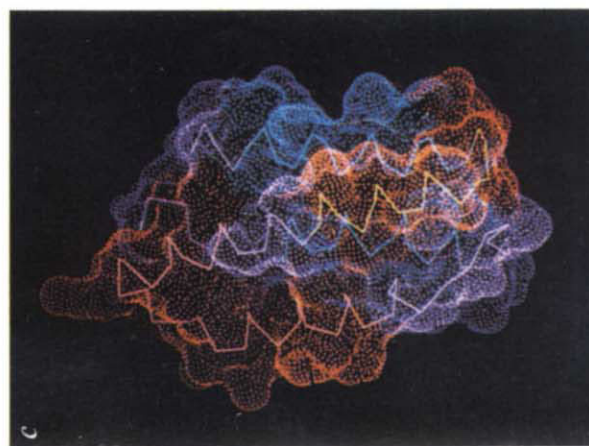
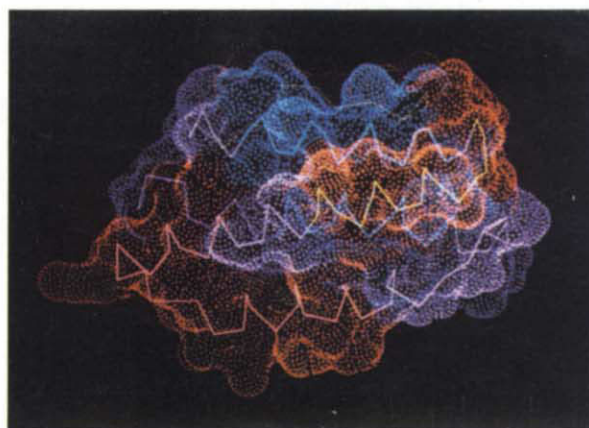
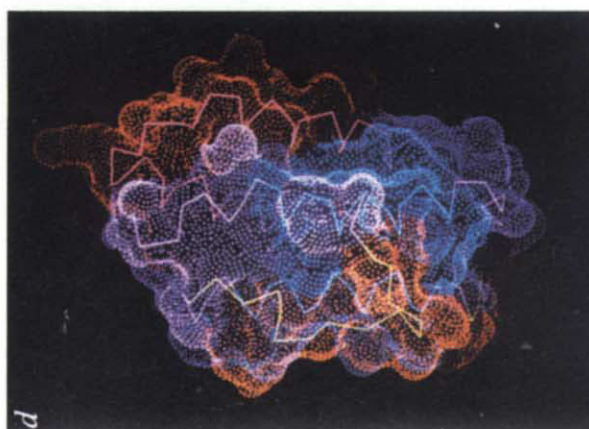
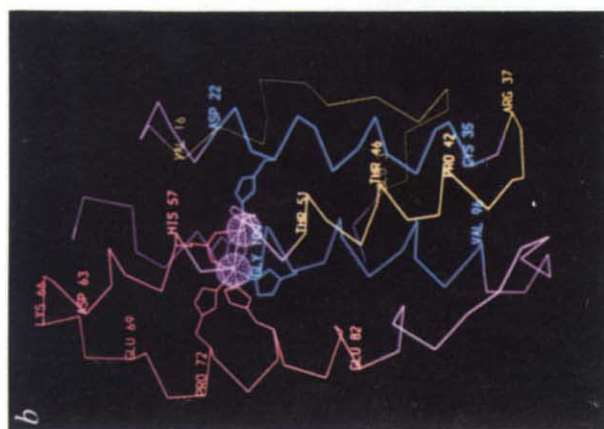
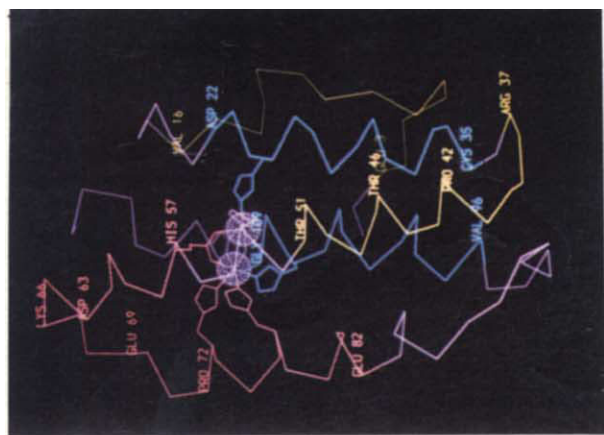
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To study the nature of antigenic recognition, antibodies have been prepared against a set of peptide sequences representing both highly mobile and well-ordered regions of myohaemerythrin, based on X-ray crystallographic temperature factors. Anti-peptide antibodies against highly mobile regions react strongly with the native protein; anti-peptide antibodies from well-ordered regions do not. Mobility is a major factor in the recognition of the native protein by anti-peptide antibodies; this may be of general significance in protein-protein interactions.

ANTIBODIES against short chemically synthesized peptides are of great use in detecting proteins and studying their structure¹. The high frequency with which these antibodies react with intact folded proteins has raised several interesting theoretical issues; one of these is addressed by considering the differences between antibodies made against intact proteins and anti-peptide antibodies. The preparation of anti-protein antibodies involves the

injection of one of the most conformationally restricted states of the molecule, the folded protein; the resultant antibodies are tested for reactivity against proteins or peptides in a state of equal or less order. The binding of these antibodies is therefore sensitive to perturbations of protein structure such as fragmentation or denaturation². Conversely anti-peptide antibodies are made against a conformationally less restricted protein site and



then tested against a protein molecule which is almost always more ordered.

To explain why antibodies to short, flexible peptides frequently react with more ordered molecules, we have considered models involving local disorder in proteins^{3,4}. We envision that sites in proteins move through a range of conformations and bind to anti-peptide antibodies only when a conformation is displayed which the antibody recognizes. Thus the antibodies act as a 'sink', trapping particular local conformations in mobile areas of proteins. Here we test this model's prediction that mobile sites in proteins will be recognized preferentially by anti-peptide antibodies.

Differential mobility

Refined high resolution X-ray crystal structures of molecules identify atomic positions and provide empirically determined information about atomic mobility in the form of the atomic temperature factors defined by $8\pi^2 u^2$, where the mean square displacement u^2 is averaged over time and the crystal lattice⁵. Patterns of temperature factors can reveal the lower-frequency concerted motions of groups of atoms, indicating the relative conformational variability of different regions^{6,7}; high factors indicate shallow potential wells (low energy barriers between

different conformations) allowing access to multiple conformations at biological temperatures. Individual atomic temperature factors are named for the temperature dependence of the velocity of atomic motion and the corresponding thermal energy available for surmounting these conformational potential energy barriers, rather than for local temperature differences within the molecule. (For simplicity, we use the suggestive terms 'hot' and 'cold' to refer to highly mobile (high temperature factors) and well-ordered regions (low factors), respectively.) Temperature factors may also reflect static disorder in the crystal, error in the absorption corrections, improper scaling and errors in the interpretation of the electron density. By examining the overall pattern of atomic temperature factors for a highly refined protein structure which has been solved independently in different crystal systems, the differential mobility of various regions of the molecule can be isolated from these other factors⁸.

To characterize patterns of temperature factors on protein surfaces, we examined 22 X-ray structures available from the Brookhaven Protein Data Bank⁹. We mapped temperature factors onto the solvent-accessible molecular surfaces¹⁰ and found them to be characterized by areas of both high and low mobility. Regions of low mobility were often, but not always, associated with interfaces between subunits in multimeric proteins or between adjacent molecules in the crystal lattice; such contacts were characterized by a lower than average ratio of side-chain to main-chain temperature factors. Otherwise, the average main-chain and atomic temperature factors also reflected the expected relative mobility of different parts of the molecular surface based on the constraints from underlying stereochemical interactions.

The differential mobilities of protein surfaces suggested to us that crystallographic temperature factors might be relevant in antibody-antigen recognition, and that this could be examined experimentally with anti-peptide antibodies. To select a protein for this study, we compiled a database of crystal structures known at sufficient resolution to provide accurate parameters of thermal mobility. We then eliminated proteins with considerable sequence homology to rabbit proteins (thus avoiding immunological tolerance) and proteases (which could complicate the immunoassays). We chose myohaemerythrin from the remaining protein structures for its well-refined temperature factors, wide range of mobility at the molecular surface and contiguous regions of the amino acid sequence characterized by either high or low mobility.

Myohaemerythrin belongs to the haemerythrin family of oxygen-carrying proteins found in some invertebrates. The structural fold for this family of proteins is an antiparallel bundle of four helices¹¹ (A, B, C and D) surrounding a two-iron centre at the active site, with a loop region at the N-terminus and shorter loops between the helices and at the C-terminus. Three-dimensional crystal structures with temperature factors have been refined at high resolution for monomeric myohaemerythrin from the marine worm *Themiste zostericola* (S. Sheriff, W.A.H. and J. L. Smith, unpublished results) and octameric haemerythrin from *Themiste dyscrita*¹². The structure of myohaemerythrin is known to 1.7 Å resolution in two crystalline directions (*a* and *c*) and to 1.3 Å in the third direction (*b*), with a crystallographic residual error of 0.159 and a r.m.s. deviation from ideal bond lengths of 0.017 Å. The related structure of haemerythrin is known to a resolution of 2.0 Å, with a residual error of 0.175 and a r.m.s. deviation from ideal bond lengths of 0.026 Å. The temperature factors for these two crystal structures are unique in being empirically corrected for packing contacts⁴⁵. Myohaemerythrin (Fig. 1*a*) demonstrates variable surface mobility of different intra-domain structural elements typical of high resolution protein structures. Before adjustment for the effects of crystal contacts, the A and D helices are relatively highly ordered, the C helix is highly mobile and the B helix varies from being relatively well ordered at the N-terminal end to relatively mobile at the C-terminal end. The 'glowing coal' temperature scale (Fig. 1*a*) delineates clearly this partitioning of the exposed surface of myohaemerythrin into regions of high and low mobility.

- **Fig. 1** Stereo pairs showing the α -carbon backbone and the molecular surface of myohaemerythrin colour-encoded by temperature factor. The molecular surface calculation^{25,32} rolls mathematically a water-sized probe sphere (1.4 Å radius) representing a solvent water molecule over the van der Waals' surface of the protein. All molecular surface calculations used individual van der Waals' radii that included implicit hydrogen atoms^{33,34}. Using the general purpose graphics modelling language GRAMPS³⁵ and the molecular modelling program GRANNY³⁶, the protein skeletal model together with its associated molecular surface (shown as a transparent set of colour-coded dots) was displayed interactively using vector computer graphics. In these figures, the terms front and back refer to the stereo directions towards and away from the reader, respectively, while all other directional terms refer to locations within the plane of the page. **a**, Patterns of mobility on the molecular surface of myohaemerythrin. This stereo view shows the solid external molecular surface colour-coded by the average main-chain temperature factors using a radiating-body colour scale. The molecule is oriented with the helices vertical, and the two-iron centre towards the top (*b* shows the skeletal model in the same orientation). The darkest colours (lowest temperature factors) are associated with the A helix (lower right) and the brightest colours (highest temperature factors) with the C helix (upper left). In these temperature factors, before adjustment for the effect of crystal contacts, the B helix (centre) varies from low (bottom, centre) to high (top, centre) temperature factors. The low temperature factors in the B helix apparently result from crystal packing contacts. This raster colour graphics representation was calculated using RAMS³⁷ and displayed on an AED raster graphics display. **b**, The synthetic peptide positions shown on the myohaemerythrin α -carbon backbone. Peptides are coloured to identify hot (red), cold (blue) and hot/contact (yellow) types of mobility, (magenta, not studied). The iron ions are shown by magenta spheres and bonds. The side chains of the iron liganding residues (His 25, His 54, Glu 58, His 73, His 77, His 106 and Asp 111) are colour-coded to match the peptide sequences in which they occur. Residues at the end of each non-nested peptide are labelled at their respective α -carbons. Hot peptides 57–66, 63–72, 69–82, 73–82 appear upper left, cold peptides 22–35, 26–35 right, 96–109, 100–109 at the centre (back), hot/contact peptides 3–16, 7–16 at the right (back) and 37–46, 42–51 at the centre (front). **c**, The location and exposed molecular surface of peptides sequences shown in the same orientation as *a* and *b*. The molecular surface and α -carbon backbone of the peptides are colour-coded to identify four categories of peptides: highly mobile in the crystallographic temperature factors (red backbone, molecular surface), highly mobile in the temperature factors adjusted for crystal contacts (yellow backbone, red molecular surface), relatively well ordered (blue backbone, molecular surface) and peptides not studied (magenta backbone, molecular surface). The peptides studied all show large amounts of exposed surface area. **d**, The location and exposed molecular surface of peptide sequences as described for *c*, but shown in the opposite orientation.

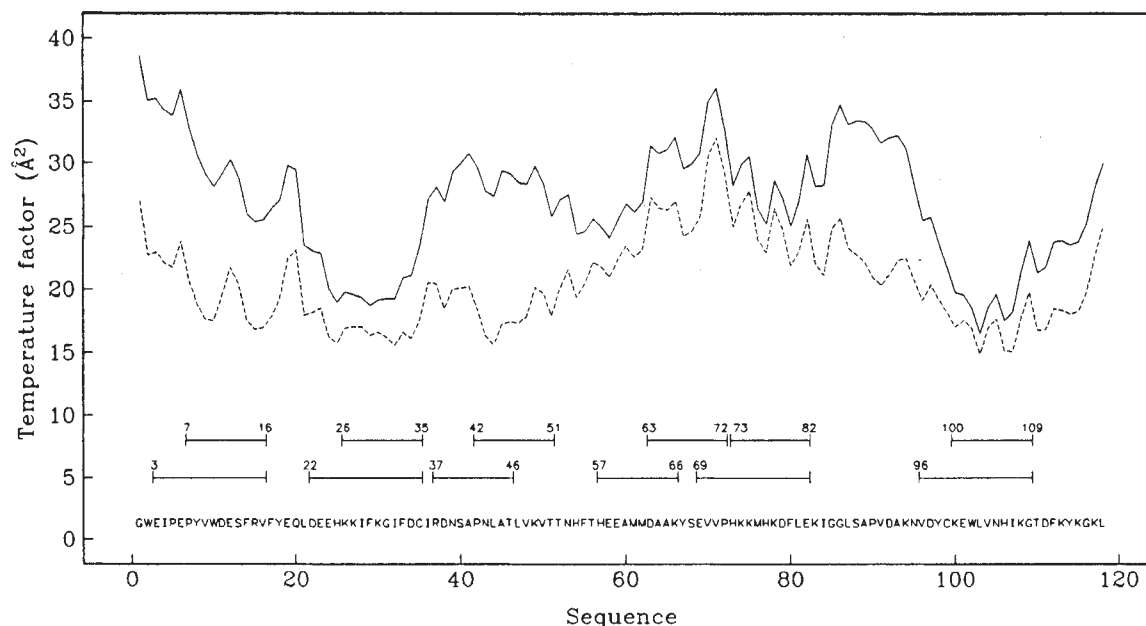


Fig. 2 Plot adapted from Sheriff *et al.*⁴⁵ of the average main-chain temperature factors along the polypeptide chain before (dashed line) and after (solid line) adjustment for crystal contacts. Regions 1–17 (N-terminal loop), 38–50 (N-terminus of the B helix), and 85–94 (C–D loop) show the greatest increase in relative mobility when corrected for crystal contacts. The 118 residue amino acid sequence of myohaemerythrin³⁸, corrected by a sequence inversion of residues 34 and 35 revealed by X-ray crystallographic studies (J. L. Smith and W.A.H., unpublished results), is shown in one-letter code (A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine). 10 and 14 residue sequences of the protein chosen for peptide synthesis are indicated by sequence-numbered bars. To allow coupling to the immunological carrier protein, an additional cysteine residue not found in the primary sequence was synthesized at the C-terminus of each peptide. Additionally, cysteine 35 in peptides 22–35 and 26–35 was replaced with a serine to avoid multiple coupling to the carrier, while maintaining peptides of a fixed length.

Methods: Peptides were synthesized as described previously³⁹ by the solid phase method^{40,41} using a Beckman model 990B peptide synthesizer. The initial amino acid resin was prepared by esterification of Boc-(MeoBzl)-Cys-OH (2.0 g anhydrous-caesium salt, 4.3 mequiv.) to chloromethylated resin (polystyrene/1% divinylbenzene, bio-beads S.X.-1, 200–400 mesh, 0.70 mmol g⁻¹) in 25 ml anhydrous dimethylformamide (DMF) by stirring for 24 h at 50 °C. A threefold excess of caesium salt over active chloride polymer gives almost complete substitution. Substitution was determined to be 0.55 mequiv. g⁻¹ by the picric acid test and amino acid analysis, and 0.53 mequiv. g⁻¹ following HCl-propionic acid hydrolysis. For each synthesis, 1.0 g (0.53 mequiv.) of Boc-(MeoBzl)-Cys resin was used. The following side-chain protecting groups were used: *O*-Br-Z for lysine and tyrosine; *O*-benzyl for threonine, serine, aspartic acid and glutamic acid; tosyl for arginine; *N*-formyl for tryptophan; *N*-dinitrophenyl for histidine. Protected amino acids (Vega Biochemicals or Peninsula Laboratories) were recrystallized from the appropriate solvent to give single spots on TLC (chloroform/acetic acid = 15:1 or chloroform/methanol = 10:1). All couplings were carried out using a 10-fold excess of Boc-AA-OH. For asparagine and glutamine, an equimolar amount of *N*-hydroxybenzotriazole was added to the protected amino acid and DMF was used as the solvent. All coupling reactions were ≥99% complete by the picric acid test. The removal of dinitrophenyl from histidine-containing peptides was accomplished with *N*-methyl mercaptoacetamide⁴² in DMF by mixing with the resin-bound peptide for 15 min. Removal was >95%. The resin bound protected peptides (500 mg to 2.5 g, 0.12–0.60 mequiv.) were treated with twice their weight of anisole and with 40 times their volume (relative to weight) of anhydrous HF at 4 °C for 1 h. For peptides containing glutamic acid, 5% v/v pyridine was added to the HF to prevent anisylation. After evaporating the HF with a stream of N₂, the red/yellow residue was mixed with anhydrous ether (50 × w/v) and stirred vigorously for 15 min; after repeating the ether extraction three times, the white residue was filtered and dried *in vacuo*. This dried resin-peptide mixture was extracted with 5% HOAc, lyophilized and amino acid analysis performed (~90% removal of the peptide from the resin). For peptides containing tryptophan, the *N*-formyl protecting group was removed from the peptide by treatment for 1.5 min at pH 11.5 (NaOH) in the presence of hydrazine. The amino acid analysis was within 5% of the theoretical value for all peptides. All peptides were coupled²⁷ to the immunogenic carrier protein KLH through the C-terminal cysteine of the peptide using *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) as the coupling reagent⁴³. Two rabbits were immunized with each peptide in a course of 3 injections of 200 µg peptide-coupled KLH (in complete Freund's adjuvant (1:1) subcutaneously on day 0, in incomplete Freund's adjuvant (1:1) subcutaneously on day 14, and with 4 mg of alum interperitoneally on day 21.)²⁷. Rabbits were bled 10 days after the third injection and boosted 3 months from the last injection and bled again 7 and 14 days later.

Myohaemerythrin peptides

Peptides, representing both helical and non-helical regions of myohaemerythrin, were selected on the basis of main-chain temperature factors averaged by residue, because the main-chain mobility reflects the global conformational variability of the protein (Fig. 1b). Three of the hot peptides include residues liganding the irons; in the native protein the mobility of these sequences is limited to conformations that do not disrupt the metal-ligand geometry.

Figure 1b–d shows the locations and exposed surfaces of the synthesized peptides in the protein. Of the exposed molecular surface area of myohaemerythrin (5,846 Å²), our synthesized peptides account for 4,005 Å² or ~69%. The four peptides synthesized for each of three categories encompass roughly

equal parts of the protein surface area: 23.0% hot, 19.3% cold and 26.2% hot/contact (hot after correction for crystal contacts), and all peptides have large areas of exposed molecular surface available for interaction with antibodies (Fig. 1c, d). Highly exposed amino acid residues tend to be less hydrophobic and, due to their external, relatively unconstrained position, more highly mobile than internal residues^{13,14}. We chose peptides to minimize differences in average exposed area and hydrophobicity per residue. Although there is some correlation of exposed area and mobility among the selected peptides, this is much stronger for side-chain than for main-chain atoms (Table 1). In fact, peptides with very similar average exposed areas may either be cold (peptides 22–35, 26–35 and 96–109) or hot (7–16, 42–51 and 57–66). There is very little correlation between temperature factor and average hydrophobicity per residue for the peptides chosen (Table 1).

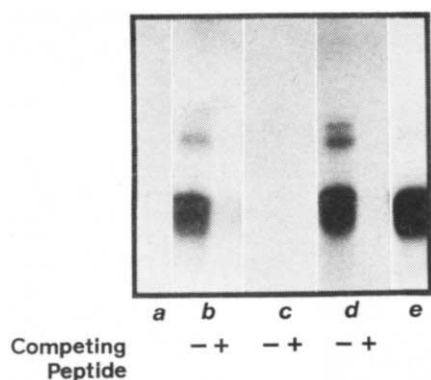


Fig. 3 Comparison of the anti-protein reactivities of antibodies raised against hot, cold, and hot/contact peptides. Lanes are representative polyacrylamide gel electrophoresis of the immunoprecipitates in the presence (+) and absence (-) of competing peptide. Lane *a*, control normal rabbit serum; lane *b*, hot peptide 57-66; lane *c*, cold peptide 26-35; lane *d*, hot/contact peptide 3-16; lane *e*, intact myohaemerythrin. Radiolabelled pellets from the immunoprecipitation were dissolved in 50 μ l of sample buffer⁴⁴ containing 2% SDS, 5% 2-mercaptoethanol and 0.001% bromophenol blue, boiled for 3 min, centrifuged to remove *S. aureus*, subjected to electrophoresis on 12.5% SDS gels, and autoradiographed. Faint bands representing aggregated states of myohaemerythrin can also be seen.

The sequence location and identity of the synthesized peptides is overlaid on the plot of the average main-chain temperature factors in Fig. 2. The 12 peptides synthesized include 83 of the total 118 residues or ~70% of the myohaemerythrin sequence. The clear division of the peptides into highly mobile and well-ordered categories is apparent in the adjusted temperature factors: two of the helices are relatively highly ordered, whereas the remainder of the structure shows significantly more mobility.

Antibody-reactive regions

To elucidate the role of molecular surface mobility in the recognition of the native protein, we raised polyclonal antibodies against synthetic peptides with each selected sequence (Table 2). Polyclonal antibodies enabled us to sample the entire range of allowed conformations for the injected synthetic peptide, whereas monoclonal antibodies would select single, possibly atypical conformations of each peptide.

The anti-peptide antisera were originally assayed for reactivity against the homologous immunizing peptide by enzyme-linked immunoabsorbent assay (ELISA). All but one of the synthetic peptides are immunogenic (Table 2); their relative immunogenicity is not correlated with their mobility. We next assayed the anti-peptide antisera for reactivity with myohaemerythrin (Table 2). All antisera raised against cold peptides (22-35, 26-35, 96-109 and 100-109) gave negative or low reactivity with the protein, whereas all antisera raised against hot peptides (excluding peptide 42-51, which did not raise any antibodies) gave higher reactivity against the protein. Of the antisera against hot peptides, those with the lowest myohaemerythrin reactivity (57-66 and 63-72) also had the lowest reactivity against their respective homologous immunizing peptides.

We used the method of Farr¹⁵ to estimate the avidity or effect of dilution on the binding of anti-peptide antisera to myohaemerythrin at five concentrations (1, 5, 10, 50 and 100 pmoles). The relative averaged avidity of the anti-peptide antisera against the protein (peptides 3-16 > 7-16 > 37-46 > 57-66 > 63-72, 69-82, 73-82, 96-109) matched the pattern of relative reactivity, antisera with higher titres generally showing greater avidity. (The negative or low reactivity of the remaining anti-peptide antisera with the protein precluded measurement of their avidities.) ELISA analyses may involve some denaturation of the target protein. We therefore assayed immunoprecipitation in conditions preserving the native structure of myohaemerythrin. Retention of the characteristic visible spectrum with a 338 nm peak¹⁶ (due to the iron centre) verified that the ligand environment remains unchanged. The results of these immunoprecipitation studies and the associated gel electrophoresis analysis (Table 2, Fig. 3) are consistent with the ELISA analyses.

To confirm the specificity of the anti-peptide antisera, we also performed immunoprecipitation studies in the presence of homologous and heterologous peptides (Table 2, Fig. 3). The anti-myohaemerythrin reactivity of antisera raised against the hot peptides was strongly inhibited by incubation with the corresponding homologous peptide, whereas incubation with the irrelevant heterologous peptide (synthesized from a sequence for influenza virus haemagglutinin) did not inhibit anti-myohaemerythrin reactivity, thus confirming the specificity of the antisera. Interestingly, antisera against cold peptides that showed low reactivity with the protein (22-35, 96-109) were, at most, only slightly inhibited by incubation with the corresponding homologous peptides. This suggests that the anti-myohaemerythrin reactivity of antibodies raised against these

Table 1 Myohaemerythrin peptide data

Sequence position	Secondary structure	Mobility				Average exposed area	Average hydrophobicity
		Type	Main*	Main	Side*		
3-16	N-terminal	Hot*	30.4	19.9	36.2	52.3	0.02
7-16	N-terminal	Hot*	28.6	18.8	32.4	43.8	0.02
22-35	A-helix	Cold	20.4	16.8	26.2	40.6	-0.16
26-35	A helix	Cold	20.1	16.6	26.2	43.8	0.05
37-46	A loop	Hot*	28.9	18.5	30.7	59.7	-0.23
42-51	B helix	Hot*	28.4	17.8	31.8	44.9	0.26
57-66	B helix	Hot	28.0	24.2	30.2	45.6	-0.11
63-72	B-C loop	Hot	31.9	27.4	34.1	60.0	0.05
69-82	C helix	Hot	29.6	26.2	35.9	54.9	-0.18
73-82	C helix	Hot	27.9	24.9	35.2	55.4	-0.41
96-109	D helix	Cold	20.8	17.6	26.7	40.1	0.04
100-109	D helix	Cold	19.4	16.9	25.1	36.7	-0.01

Peptides are numbered by their respective sequence position in myohaemerythrin. All mobilities are derived from the temperature factor data for the intact protein⁴⁵. Peptides are classified into three types of mobility: hot (high average main-chain temperature factors), cold (low average main-chain temperature factors*) and hot* (hot/contact, high average main-chain temperature factors after correction for the effects of crystal contacts). Mobility in $\text{\AA}^2$ is expressed as average main chain* (calculated from the atomic temperature factors for N, C α , C, O atoms adjusted for crystal contacts), average main chain (calculated from the atomic temperature factors for N, C α , C, O atoms), and average side chain* (calculated from the atomic temperature factors for side-chain atoms adjusted for crystal contacts). Average exposed area in $\text{\AA}^2$ is calculated using the molecular surface program MS²⁵. Average hydrophobicity is calculated using the Eisenberg consensus scale²⁶. Properties were first averaged for each residue, then these residue averages were averaged for each peptide.

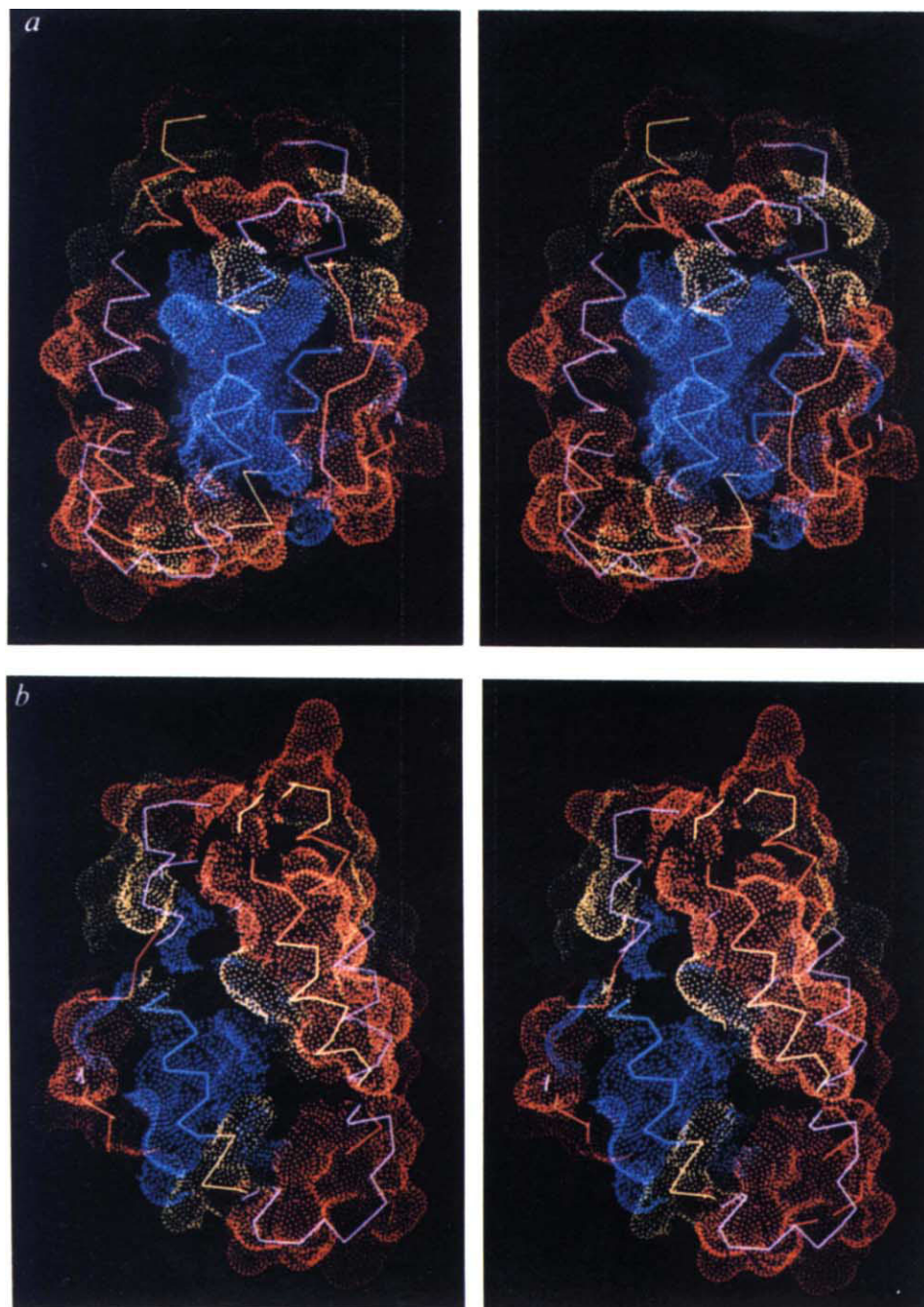


Fig. 4 Stereo views showing the correlation between the mobility of sites in a protein and their reactivity with anti-peptide antibodies. The α -carbon backbone is colour-coded by antigenic reactivity based on the immunoprecipitation results (Table 2): red, high ($>3,500$ c.p.m.), yellow, medium (2,000–3,000 c.p.m.), blue, low (<200 c.p.m.), magenta, not studied. The exposed molecular surface is colour-coded by average adjusted main-chain temperature factors (Table 1): red, hot ($\geq 27 \text{ \AA}^2$), yellow, medium (22–27 $\text{ \AA}^2$), blue, cold ($<22 \text{ \AA}^2$). To identify the portions of the molecular surface associated with each synthesized peptide sequence, the myohaemerythrin molecule is dissected into individual peptides (backbone and associated molecular surface) shown in a slightly exploded view. Since the peptides were coupled onto the KLH carrier at the C-terminal amino acid, overlapping and nested peptide sequences were assigned the level of reactivity associated with the sequence of the N-terminal portion of the relevant peptide. *a*, The following peptide regions (correspondence of secondary structure descriptions to sequence positions defined in Table 1) show the correlation of their temperature factors with antigenic reactivity: the N-terminus (red backbone and surface, lower right), the A helix (blue backbone and surface, centre), the A loop (red backbone and surface, lower left, front) and the C-terminal end of the B helix (red and yellow backbone and surface, upper left). *b*, The relationship is apparent for the remaining peptide regions: the non-overlapping fragment of the B-C loop (yellow backbone and red surface, top), the C helix (red and yellow backbone and surface, upper right), and the D helix (blue and yellow backbone and surface, lower left).

cold peptides may largely represent either nonspecific binding or low avidity.

The striking correlation seen in both types of immunoassay between the mobility of sites in a protein and their reactivity with anti-peptide antibodies is seen when the reactivities of the anti-peptide antibodies are mapped onto the α -carbon backbone and the average adjusted main-chain temperature factors mapped onto the external molecular surface (Fig. 4). Of the cold peptides, only antibodies to peptide 96–109 have reactivity with intact myohaemerythrin; this peptide has four residues with higher temperature factors on its N-terminal end. Of the hot peptides, the N-terminal loop peptide 3–16 represents the target site with the highest mobility and reactivity with anti-peptide antibodies. Reduced mobility due to structural constraints in myohaemerythrin resulting from the metal liganding may modulate the degree of antigenic reactivity. In the immunoprecipitation assays, antisera against the shorter of

the two nested peptides containing two metal ligands in the intact molecule (73–82) have the lowest anti-protein reactivity of any hot peptide antisera. These results support the correlation between mobility and reactivity with anti-peptide antibodies.

Part of the effect seen in these experiments could be the result of differences in the level of antibody raised against the individual peptides. Our basic findings remained the same when reactivities were normalized as a function of the anti-peptide titres: thus expressed, the anti-protein reactivities improved for antisera against hot peptides and changed little for those against cold peptides. Furthermore, we found that the increase in anti-peptide titres after the rabbits received booster injections of peptides led to increases in anti-myohaemerythrin reactivity for the antibodies raised against the hot peptides, compared with little or no increase in reactivity for the antibodies raised against the cold peptides (data not shown).

Conclusions

The ability of anti-peptide antibodies to recognize their corresponding intact folded proteins is best understood as a dynamic process which is most favoured when the conformation of the peptide immunogen approximates the target site in the protein and that site is mobile. The relative contributions of each factor to the success of an immunogen are difficult to assess and may vary from peptide to peptide. Our data suggest that recognition involves the interaction of antibodies with sites in the protein which can adopt multiple conformations that may differ from the average structure seen by X-ray crystallography. One may envision a process in which the initial interaction with the antibody is sufficient for recognition of the appropriate site, after which considerable induction of shape may take place.

Table 2 Reactivity of anti-peptide antisera

Sequence position	ELISA titres		Immunoprecipitation	
	α -Peptide vs peptide	α -Peptide vs myohaemerythrin	125 I-Myohaemerythrin precipitated (c.p.m.)*	% Inhibition by peptide†
3-16	640-1,280	12,800	14,849	97.1
7-16	640-1,280	3,200	7,384	92.6
22-35	1,280-2,560	—	180	0.0
26-35	320-640	60	0	0.0
37-46	640-1,280	9,500	4,026	100.0
42-51	—	—	0	0.0
57-66	160-320	600	7,277	80.0
63-72	160-320	375	2,543	77.6
69-82	>2,560	1,050	3,774	91.3
73-82	>2,560	1,400	2,154	62.0
96-109	1,280-2,560	200	2,617	22.5
100-109	320-640	—	0	0.0

Reactivities of the various anti-peptide antisera raised in pairs of rabbits were assayed against the antigen in the solid phase using ELISAs and against the antigen in solution using immunoprecipitation. ELISA titres are expressed as the reciprocal of antibody dilution extrapolated to bind 50% of 50 pmol antigen per well. ELISA: Assays followed the procedure described by Green *et al.*²⁷ 50 pmole antigen (synthetic peptide or myohaemerythrin molecule) in phosphate-buffered saline (PBS, 10 mM sodium phosphate, 0.15 M NaCl, pH 7.2) were added to each well of a polystyrene microtitre plate and dried overnight at 37°C. The dried antigen was then fixed to the plate by treatment with 50 μ l of methanol per well for 5 min at room temperature. The plates were coated for 4 h with a solution of non-fat dry milk (BLOTTO, ref. 28) to block nonspecific absorption of antibodies to the plate. Following the addition of 25 μ l antiserum (serially diluted with the same solution) to each well, the plates were incubated overnight at room temperature and the unbound antibody was washed off with water. The antigen-antibody complex was reacted with goat anti-rabbit IgG coupled to glucose oxidase²⁹. After removal of excess goat anti-rabbit IgG with water, this sandwich complex was measured enzymatically by the addition of enzyme substrate. For less denaturing (wet plate) ELISAs, the antigen was attached to the plates by overnight incubation in 15 mM saline in 10 mM phosphate buffer, pH 9.0. Non-immunogenic peptide 42-51 continued to give negative results in repeated ELISA experiments. Rabbit antisera against the intact myohaemerythrin protein were tested by ELISA for reactivity with the protein and with the synthetic peptides. The anti-protein antisera showed high reactivity (titres >1,280) against myohaemerythrin, but did not react with any of the tested synthetic peptides. Controls showed no cross-reactivity between kehole limpet haemocyanin (KLH) and myohaemerythrin and no anti-myohaemerythrin reactivity in preimmune serum. Immunoprecipitation: Myohaemerythrin was radiolabelled with 125 I by the chloramine-T reaction³⁰ to a final incorporation of about 25 μ Ci μ g⁻¹. After dialysis, 10⁶ c.p.m. of 125 I-myohaemerythrin in PBS were reacted with 10 μ l of anti-peptide antiserum for 60 min on ice. The antigen-antibody complexes were precipitated with formalin-fixed *Staphylococcus aureus*. The pellets were washed once with RIPA buffer (PBS containing 1% Nonidet P-40, 0.05% sodium deoxycholate and 0.1% SDS) and twice with 500 mM LiCl, 100 mM Tris (pH 8.5), then analysed for radioactivity. In peptide competition experiments, the anti-peptide antibodies were preincubated for 60 min with 100 μ g peptide in PBS before the addition of 125 I-myohaemerythrin. Western blot analysis: The correlation of mobility with antigenic reactivity was also studied by Western blot analysis³¹. Following polyacrylamide gel electrophoresis of myohaemerythrin in denaturing conditions (the samples were boiled in the presence of 2% SDS and 5% 2-mercaptoethanol), the protein was transferred to a nitrocellulose filter and incubated with selected anti-peptide antisera. Detection of the bound antisera with 125 I-labelled *S. aureus* protein A showed that the antisera against cold peptides did not react well with myohaemerythrin in these conditions, whereas antisera against hot peptides showed relatively strong reactivity.

* Average of two independent experiments, after correction for nonspecific binding.

† 100.0 % minus per cent of activity remaining in the presence of the peptide.

Mobility is thus involved in both adoption of protein conformations suitable for recognition by the antibody and the degree to which a given region can be induced into a conformation that fits the antibody binding site.

For proteins in solution, the range and extent of mobility are probably greater than those calculated from the average main-chain temperature factors; dynamics calculations indicate that conformational flexibility is greater than that seen in the X-ray crystal structures¹⁷. In myohaemerythrin, the highest temperature factors predict average isotropic displacements between ± 0.7 and ± 0.9 Å for the main and side chains, respectively. Rather than defining exactly the excursions of individual residues¹⁸⁻²⁰, however, these high values identify areas with relatively low energy barriers between alternate conformations. The overall mobility does not imply any complete local unfolding in myohaemerythrin, as no visible spectroscopic change in the ligand environment of the two iron atoms occurs in the solvent and salt conditions used in the present study. The fact that strong protein-reactive antibodies can be induced by peptides representing highly mobile sites in the protein that contain iron binding residues (Fig. 1b) indicates that mobility, but not complete local disorder, is important for antigenic reactivity.

The anti-protein reactivity of antibodies to synthesized myohaemerythrin peptides correlates better with the mobility of the target sites than with their exposed surface area. The cold peptides 22-35, 26-35 and 96-109 have similar average exposed surface areas to hot peptides 7-16 and 57-66, but much less reactive antisera. Thus, the apparent relationship between exposed area and antigenic reactivity²¹ may actually reflect the increased mobilities of turns, loops and other highly exposed areas. As a large percentage of the exposed molecular surface (80% in myohaemerythrin) belongs to highly mobile residues, our results are consistent with studies¹ showing that most anti-peptide antibodies react with the corresponding intact protein.

Protein-protein interaction is a complex process involving many characteristics of the molecular surface, including shape complementarity and hydrophobicity of the interacting molecular surfaces²². The mapping of electrostatic potential onto molecular surfaces^{23,24} has revealed the importance of electrostatic forces in intermolecular interactions. Our present results showing a correlation between the mobility of sites in a protein and their reactivity with anti-peptide antibodies suggest that molecular mobility is an essential part of the antigenic recognition process and perhaps of protein-protein recognition in general. The distinguishing feature of anti-peptide antibodies is their predetermined specificity. Elucidation of the amino acid sequence of a protein should allow the use of site-specific anti-peptide antibodies for the investigation of the role of conformational flexibility in protein-protein recognition processes.

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LETTERS TO NATURE

Near-infrared observation of the circumsolar dust emission during the 1983 solar eclipse

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A near-infrared excess emission superposed on the coronal continuum at $\sim 4R_{\odot}$ from the Sun was first observed at $2.2\text{ }\mu\text{m}$ by Peterson¹ and MacQueen² during the total solar eclipse in 1966, and was later confirmed by MacQueen² using a balloon-borne coronagraph. Two subsequent observations were made; one³ obtained a similar result, while the other⁴ detected no excess emission. The excess emission was thought to be due to thermal radiation by a dust cloud around the Sun, and interpreted to be interplanetary dust². Brecher *et al.*⁵, on the other hand, have proposed that the ring is composed of much larger objects (of about 10 km diameter). To obtain more definite information, we have therefore carried out observations of near-infrared brightness distributions of the solar corona, using a balloon-borne photometer at a balloon altitude, during the total eclipse on 11 June 1983 in Indonesia. As we report here, emissions in excess of the strong coronal background emission were recorded in some of the scans at $\sim 4R_{\odot}$ from the Sun. The spatial distribution of the excess emission implies the existence of a circumsolar ring of dust lying approximately in the ecliptic plane.

The balloon-borne instrument we used for near-infrared photometric scanning consisted of a 16-cm Cassegrain telescope and a simultaneous four-band photometer in a reaction wheel-stabilized gondola, together with a high-sensitivity television camera. The photometer had four 4-element PbS detector arrays thermoelectrically cooled at separated foci with three beam-splitters. The infrared bands investigated were $1.25\text{ }\mu\text{m}$ ($\Delta\lambda = 0.24\text{ }\mu\text{m}$), $1.65\text{ }\mu\text{m}$ ($\Delta\lambda = 0.23\text{ }\mu\text{m}$), $2.25\text{ }\mu\text{m}$ ($\Delta\lambda = 0.28\text{ }\mu\text{m}$) and $2.8\text{ }\mu\text{m}$ ($\Delta\lambda = 0.7\text{ }\mu\text{m}$). The field of view of each element was $0.3R_{\odot} \times 0.3R_{\odot}$, and the arrays were orientated perpendicular to the scan path.

A $15,000\text{-m}^3$ balloon was successfully launched by a joint team of ISAS (the Institute of Space and Astronautical Science, Japan) and LAPAN (Indonesian National Institute of Aeronautics and Space) at the Watukosek Balloon Base in East Java. Virtual tracking of the eclipsed Sun at a floating altitude of 30 km was performed by rotating the magnetometer stage at an appropriate rate after the desired instrument orientation had been obtained. The attitude stability was better than 10 arc s ($\sim 0.01R_{\odot}$). Absolute positional error was $<0.2R_{\odot}$ in the scan

direction and $<0.3R_{\odot}$ in the perpendicular.

Scanning was performed by small-amplitude oscillation around the cross-elevation axis. The width of the scan was 5° ($20R_{\odot}$) and its rate was 0.05 deg s^{-1} ; this allowed $1\frac{1}{2}$ complete scans between $6R_{\odot}$ on the west side of the Sun and $14R_{\odot}$ on the east side. Scan paths crossed the ecliptic plane on the west side at $\sim 4R_{\odot}$ with an inclination of $\sim 7^{\circ}$. The configuration of the scan path and beam positions of each detector are shown in Fig. 1. In-flight and post-flight calibrations have indicated possible errors of $\pm 30\%$ in absolute brightness determination. Polarization at $2.25\text{ }\mu\text{m}$ was also measured using a rotating half-wave plate and a fixed polarizer. The result of the polarization measurement will be described elsewhere.

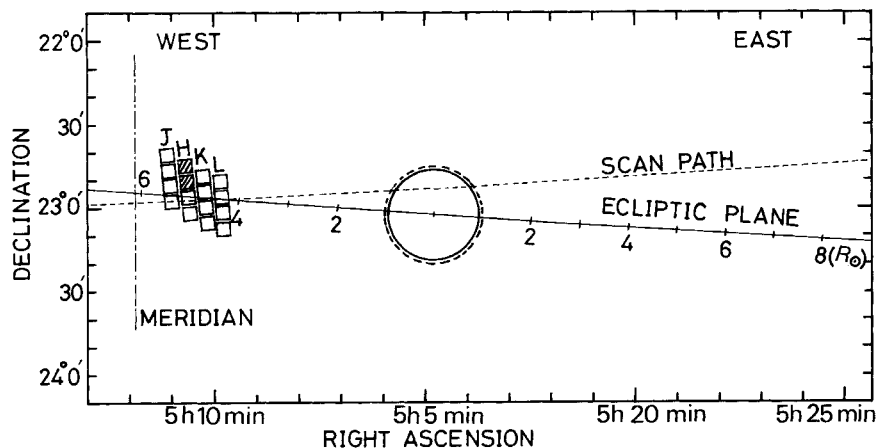
Figure 2 shows brightness distributions in each band on the west side of the Sun along the scan path. At $1.65\text{ }\mu\text{m}$, a definite excess over the smooth coronal component is seen between $2.8R_{\odot}$ and $4.5R_{\odot}$ from the Sun on the west side, showing a peak at $3.8R_{\odot}$. The brightness of the excess component at $3.8R_{\odot}$ on the west side is $6 \times 10^{-7}\text{ W cm}^{-2}\text{ }\mu\text{m}^{-1}\text{ sr}^{-1}$. A weaker excess is also seen between about $3R_{\odot}$ and $4.4R_{\odot}$ on the east side, where the peak again occurs at $3.8R_{\odot}$. The brightness of the excess component at $3.8R_{\odot}$ on the east side is $2.2 \times 10^{-7}\text{ W cm}^{-2}\text{ }\mu\text{m}^{-1}\text{ sr}^{-1}$. A relatively small but obvious excess emission is also seen in the $1.25\text{-}\mu\text{m}$ profiles from about $3.2R_{\odot}$ to $4.5R_{\odot}$ on the west side of the Sun, where scans have just crossed the ecliptic plane (see Fig. 1). The peak is again at $\sim 3.8R_{\odot}$ and the brightness of the excess component is $3.7 \times 10^{-7}\text{ W cm}^{-2}\text{ }\mu\text{m}^{-1}\text{ sr}^{-1}$. The contribution of stray light from the strong inner corona has been estimated from independent laboratory data for the off-axis rejection rate. The effect is not serious in the region further than $1.8R_{\odot}$ in $1.65\text{-}\mu\text{m}$ and $2.25\text{-}\mu\text{m}$ bands, or further than $3.0R_{\odot}$ in $1.25\text{-}\mu\text{m}$ and $2.8\text{-}\mu\text{m}$ bands. Any error due to such stray light is safely below 30% of the above excess emissions.

In the $2.25\text{-}\mu\text{m}$ band, we have failed to detect definite peaks larger than the 3σ detection limit of $1.2 \times 10^{-7}\text{ W cm}^{-2}\text{ }\mu\text{m}^{-1}\text{ sr}^{-1}$, owing to degraded sensitivity in this band. However, a small feature could be discerned at about $4R_{\odot}$ on the west side, where the brightness is $1 \pm 0.4(1\sigma) \times 10^{-7}\text{ W cm}^{-2}\text{ }\mu\text{m}^{-1}\text{ sr}^{-1}$, roughly consistent with the previous observations of Peterson¹ and MacQueen². We found no definite excess component in the $2.8\text{-}\mu\text{m}$ band at the 3σ level of $1.2 \times 10^{-7}\text{ W cm}^{-2}\text{ }\mu\text{m}^{-1}\text{ sr}^{-1}$.

Figure 3 shows a near-infrared energy distribution of the excess component obtained from the present observation, together with previous $2.2\text{-}\mu\text{m}$ data. It has a maximum at $1.65\text{ }\mu\text{m}$ and drops off at both shorter and longer wavelengths, especially between 1.65 and $2.25\text{ }\mu\text{m}$, differing significantly from the solar spectrum. Therefore, we argue that the excess emission component is not caused by scattered light but is due mainly to thermal radiation of circumsolar dust particles of appropriate composition to produce the spectrum.

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Fig. 1 The scan path of the telescope beam and the projected focal plane array of detectors. J, H, K and L' denote the 1.25-, 1.65-, 2.25- and 2.8- μm bands, respectively. Data from two detectors of the 1.65- μm band, indicated by hatched squares, were not used for data analysis, due to anomalous large noise.



Lamy has proposed⁶ that the near-infrared excess emission may be caused by silicate grains such as obsidian particles lying just outside a dust-free zone, while Mukai *et al.*⁷ have suggested a two-component model of obsidian and graphite. However, the spectral features observed by us are not consistent with these materials, even taking into account the large uncertainty in the data at longer wavelengths. One interpretation we have envisaged is that the emission in question is accounted for by thermal emission from olivine particles of the order of 100 μm in radius at a temperature of $\sim 1,300$ K. Olivine possesses a strong absorption band between 0.8 and 1.7 μm , and hence is more emissive within this band. Smaller olivine particles seem unlikely because the intensity of scattered light would obliterate the thermal radiation, making the spectrum appear solar. As an illustration, the thermal emission spectrum of olivine of radius 100 μm at a temperature of 1,300 K is shown in Fig. 3.

Our suggestion that the circumsolar dust may consist of relatively large particles composed mainly of olivine is based on recent studies of interplanetary dust and its mineralogy. Weiss-Wrana⁸ has shown that the principal properties of the zodiacal light ('the false dawn') are well represented by opaque irregularly-shaped chondritic particles in the size range 20–120 μm . Most stratospheric micro-meteorites, known as Brownlee particles⁹, have an opaque, black appearance and a composition similar to C1 carbonaceous chondrites. Furthermore, olivine

is known to be a major constituent of extraterrestrial solids such as stony meteorites. Finally, Hashimoto *et al.*¹⁰ have demonstrated, in experiments on the thermal metamorphism of C2 type carbonaceous chondrites, that the matrix (mineralogically amorphous) becomes olivine when heated in a low pressure environment to $>1,000$ K.

The infrared spectrum we have observed can thus be interpreted as being due to the thermal metamorphism of dust particles as they spiral down to the hot region of the circumsolar dust cloud. However, the orbital radius at which the emission peaks were recorded must be explained in terms of a time-dependent vaporization process of relatively large particles (of the order of 100 μm) having optical properties that are susceptible to temperature changes—ranging from opaque in distant regions to a more transparent (olivine-like) nature in nearer and thus hotter regions around the Sun. A problem concerning the equilibrium temperature of such particles as envisaged here is that, according to Röser and Staude¹¹, a 100- μm olivine particle would have a temperature of 2,000 K at $\sim 4R_{\odot}$.

The present observations also provide information about the spatial distribution of dust, although the scan paths were such that a genuine central peak on the east side of the ecliptic plane was missed. As shown in Fig. 2, the excess component of the 1.65- μm band seems to extend from $2.8R_{\odot}$ to $4.5R_{\odot}$, with a small peak at around $3.8R_{\odot}$. (These features bear some

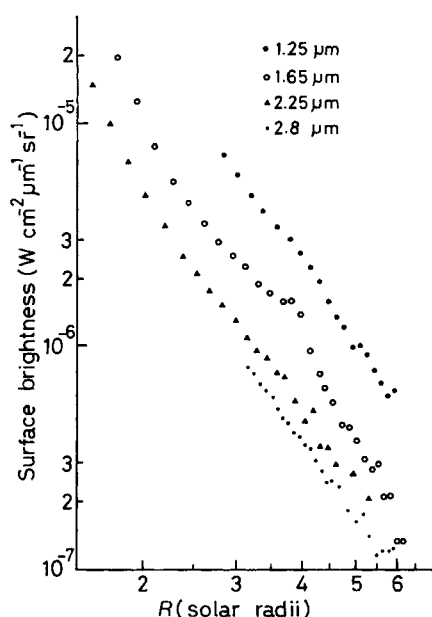


Fig. 2 Brightness distributions of each band observed on the west side of the Sun. The data are averaged over every $0.15R_{\odot}$ except for the 2.8- μm band inside $4.4R_{\odot}$, where the interval is $0.08R_{\odot}$.

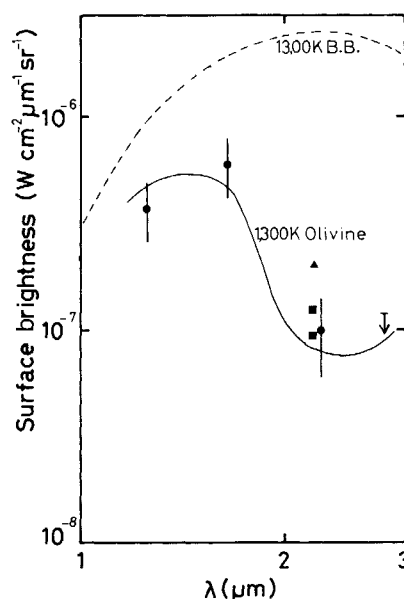


Fig. 3 Energy spectrum of the excess emission component. Previous observations at 2.2 μm are also shown: $\blacktriangle$, ref. 1; $\blacksquare$, ref. 2. The solid curve represents the thermal radiation of an olivine particle of radius 100 μm at a temperature of 1,300 K. The dashed line is a 1,300 K black body curve for comparison.

resemblance to the 2.2- μm profile found by Peterson¹², but differ in that our data show no steep trough just inside the narrow peak.) The 1.65- μm brightness also shows an excess emission between 3.0 $R_{\odot}$ and 4.4 $R_{\odot}$ in the east side scans, which are $\sim 1R_{\odot}$ from the ecliptic at 4 $R_{\odot}$, and its intensity is considerably smaller. This suggests that the circumsolar dust cloud is a ring and not a spherical shell and is consistent with the hypothesis that the dust particles responsible for the observed infrared excess emission are identical with the interplanetary dust causing the zodiacal light—which is generally assumed to be reflected by grains as large as a few hundred micrometres, probably in orbits of high ellipticity (see, for example, ref. 13).

Finally, we can make a rough estimate of the total mass of the dust cloud in the region of infrared emission. Let us assume that the cloud is formed into a doughnut shape of mean diameter 8 $R_{\odot}$, radial width 1 $R_{\odot}$ and thickness 1 $R_{\odot}$, and that the rep-

resentative radius of olivine particles is 100 μm . The observed infrared fluxes measured at 1.25 and 1.65 μm will then be related, as an order of magnitude consideration, to an average number density of 1×10^{-14} particles per cm^3 and a total dust mass of 7×10^{14} g. The spatial density obtained here is about 15 times the value of simple extrapolation based on the mass density 4×10^{-23} g cm^{-3} at 1 AU (ref. 14), applying an $r^{-1.3}$ dependence on heliocentric distance.

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New model of Saturn's ionosphere with an influx of water from the rings

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Current theoretical models of Saturn's ionosphere are similar to those of Jupiter's because of the gross similarity of their upper atmospheres. In the case of Jupiter, the theoretical models can be fitted reasonably well to ionospheric electron density profiles^{1,2} obtained from the Pioneer and Voyager radio occultation experiments^{3–5}. In contrast, the theoretical models of Saturn's ionosphere are inconsistent with both the ionospheric electron density profiles^{6,7} obtained from the Pioneer and Voyager occultation observations^{8–10} and the large diurnal variation of maximum ionospheric electron density deduced from studies of Saturn lightning discharges^{11,12}. We propose a radically different model of Saturn's ionosphere in which water plays a major role as a minor constituent present by downward diffusion from an external source. Our model Saturn ionosphere is a classical 'F2' type layer resulting from the photodissociative production of H^+ from H_2 and rapid chemical loss by a series of charge exchange reactions with water. A planet-wide influx of $\sim 4 \times 10^7$ molecules $\text{cm}^{-2} \text{s}^{-1}$ of water from the rings is consistent with the observed ionospheric electron densities. An enhanced influx of water occurs at latitudes (-38° , $+44^\circ$) connected magnetically to the inner edge of Saturn's B ring¹² where an electromagnetic erosion process¹³ takes place. Present-day influx at these latitudes may be as large as $\sim 2 \times 10^9$ molecules $\text{cm}^{-2} \text{s}^{-1}$.

The essential features of previous theoretical models of Saturn's ionosphere are summarized below. The major topside ion, H^+ , is produced via photoionization of atomic H or photodissociative ionization of H_2 . Both H and H^+ are extremely long-lived above the homopause ($\sim 1,100$ km altitude; all altitudes referenced to the 1 bar pressure level) and are removed

Table 1 Chemical reactions in Saturn's ionosphere: H_2 , H_2O , H

	Ion production	Rate*	Ref.
A1	$\text{H}_2 + h\nu \rightarrow \text{H} + \text{H}$	$4.5 \times 10^{-10} \text{ s}^{-1}$	[7]
A2	$\text{H}_2^+ + \text{e}$	5.4×10^{-10}	[7]
A3	$\text{H} + \text{H}^+ + \text{e}$	9.5×10^{-11}	[7]
A4	$\text{H} + h\nu + \text{H}^+ + \text{e}$	7.3×10^{-10}	[7]
A5	$\text{H}_2\text{O} + h\nu \rightarrow \text{OH} + \text{H}$	1.0×10^{-7}	[19]
	$\text{OH}^+ + \text{H} + \text{e}$	5.5×10^{-10}	[19]
	$\text{H}_2 + \text{O}(^1\text{D})$	1.4×10^{-8}	[19]
	$\text{H}_2\text{O}^+ + \text{e}$	3.3×10^{-9}	[19]
	$\text{H}_2 + \text{O}^+ + \text{e}$	5.8×10^{-11}	[19]
	$\text{H}^+ + \text{OH} + \text{e}$	1.3×10^{-10}	[19]
Charge exchange/recombination			
B1	$\text{H}_2\text{O} + \text{H}^+ \rightarrow \text{H}_2\text{O}^+ + \text{H}$	$8.2 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$	[18]
B2	$\text{H}_2\text{O}^+ + \text{H}_2 \rightarrow \text{H}_3\text{O}^+ + \text{H}$	6.1×10^{-10}	[18]
B3	$\text{H}_3\text{O}^+ + \text{e} \rightarrow \text{H}_2\text{O} + \text{H}$	4.3×10^{-7}	[18]
B4	$\text{H}_2 + \text{OH}$	4.3×10^{-7}	[18]
B5	$\text{H} + \text{H} + \text{OH}$	4.3×10^{-7}	[18]
B6	$\text{H}_2^+ + \text{H}_2 \rightarrow \text{H}_3^+ + \text{H}$	2.0×10^{-9}	[7]
B7	$\text{H}_3^+ + \text{e} \rightarrow \text{H}_2 + \text{H}$	1.0×10^{-7}	[7]
B8	$\text{H}^+ + \text{e} \rightarrow \text{H} + h\nu$	2.0×10^{-12}	[7]
B9	$\text{OH} + \text{H}_2 \rightarrow \text{H}_2\text{O} + \text{H}$	3.6×10^{-11}	[18]

* Photoionization rates normalized to 10 AU by $1/R^2$ scaling of reference values.

by downward diffusion to the vicinity of the homopause where appreciable concentrations of heavier gases (most importantly methane) are encountered. H^+ ion loss occurs essentially at the homopause via charge exchange and electron-ion recombinations, resulting in a peak ion concentration of $\sim 2 \times 10^5 \text{ cm}^{-3}$ at $\sim 1,200$ km altitude. This very simplified picture emerges from a consideration of many possible chemical reactions and detailed calculations taking into account the production, diffusion and chemical losses of many atmospheric constituents⁷.

Figure 1 is a representative summary of the dawn and dusk electron density profiles (as a function of altitude) obtained from analyses of the Pioneer and Voyager radio occultation data. In Fig. 1, mid-latitude Voyager observations are compared with a typical model ionospheric electron density profile. (Figure 2 shows all of the radio science observations.) The measured electron densities are all approximately an order of magnitude less than those calculated on the basis of current models.

Maximum observed ionospheric densities are $\sim 1 \times 10^4 \text{ cm}^{-3}$, compared with a calculated maximum density of $2 \times 10^5 \text{ cm}^{-3}$ in all previous models. The peak electron density is observed at between 2,000 and 3,000 km altitude instead of the calculated 1,200 km altitude. The discrepancy in the altitude of the peak is particularly significant in light of the thermal structure of the atmosphere. Above $\sim 1,600$ km, the atmosphere is isothermal, with a temperature of ~ 800 K and an atmospheric scale height of ~ 400 km. Below that the temperature decreases linearly to ~ 125 K at 1,000 km altitude where the atmospheric scale height is ~ 60 km (ref. 7). The difference in the observed and modelled altitudes of peak ion density is thus many atmospheric scale heights. The large (~ 2 orders of magnitude) diurnal variation of maximum ionospheric electron density (Fig. 3) deduced from analyses of Saturn lightning discharges^{11,12} is even more difficult to understand on the basis of current models of Saturn's ionosphere. Without an additional loss mechanism for rapid removal of the long-lived H^+ ions, no diurnal variation is expected since the time constant for loss of H^+ above the homopause is ~ 400 h that is, many planetary rotations. This is owing to the slow electron recombination reaction $\text{H}^+ + e \rightarrow \text{H} + h\nu$ at $2 \times 10^{-12} \text{ cm}^{-3} \text{ s}^{-1}$ and the large diffusion time constant below the 2,500 km altitude of the ionospheric peak.

The observational results thus require an additional and effective loss mechanism for H^+ ions, since ion production via photodissociative ionization of H_2 and photoionization of atomic H is well understood. Several possible loss processes have been quantitatively evaluated but none has proven adequate. These include losses due to an increased methane abundance which requires an unreasonably high eddy diffusion coefficient¹⁴, removal of H^+ by vibrationally excited H_2 (ref. 6), and/or the presence of large vertical drifts^{7,8}. The possibility of an interaction between the ionosphere and ring system was recognized as early as 1975¹⁵ and a suggestion involving H^+ ion loss due to the presence of OH radicals from the rings^{16,17} was made to explain the low ionospheric densities inferred from the Pioneer observations.

We propose a simple model ionosphere dominated by the removal of H^+ ions via charge exchange with water and subsequent electron recombination. Table 1 summarizes the important chemical reactions and reaction rates; the reactions involving water have been largely drawn from work relating to comet models^{18,19}. The important reactions are H^+ ion production via dissociative photoionization of H_2 (A3) and loss via the charge exchange with H_2O (B1). The H_2O^+ ion is very short lived, reacting with H_2 (B2) to produce H_3O^+ , which is then readily removed by electron recombination (B3–B5). As in previous models, the H_2^+ ions produced by photoionization of H_2 (A2) are rapidly removed via reaction with H_2 (B6) and charge exchange (B7) and are not a major topside ion. The direct photoionization of atomic hydrogen (A4) does not significantly contribute to the H^+ ion density owing to the limited availability of atomic H.

The OH produced in B4 and B5 has a short lifetime ($\tau < 1$ s) at the altitude of the peak ion density owing to a reaction (B9) with H_2 , producing H_2O and H. Thus water acts as a catalyst in the rapid removal of H^+ ; each H^+ ion removed returns, on average, 3 or 4 H atoms with no associated chemical loss of H_2O . Photodissociation of H_2O (A5) yields OH which in the presence of appreciable H_2 (B9) rapidly reforms H_2O (as would OH from any other source). There is no net loss of water due to charge exchange or photoionization. Thus the density of water above the homopause is given by²⁰

$$N_w = N_0 \exp(-9Z/H) + FH_0/8D_0 [\exp(-Z/H) - \exp(-9Z/H)]$$

where N_0 , H_0 , and D_0 are the H_2O density, the atmospheric scale height, and molecular diffusion coefficient at a reference ($Z=0$) altitude, F is the downward flux of H_2O and H is the atmospheric scale height. Assuming that the water is readily removed by freezing out just above the homopause or by mixing

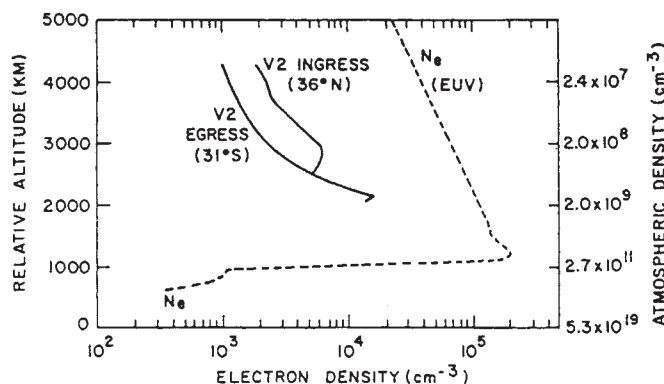


Fig. 1 Model calculations of mid-latitude ionospheric electron density as a function of altitude (extreme ultraviolet, EUV) and Voyager observations of Saturn's ionosphere at mid-latitudes, adapted from Atreya *et al.*⁷.

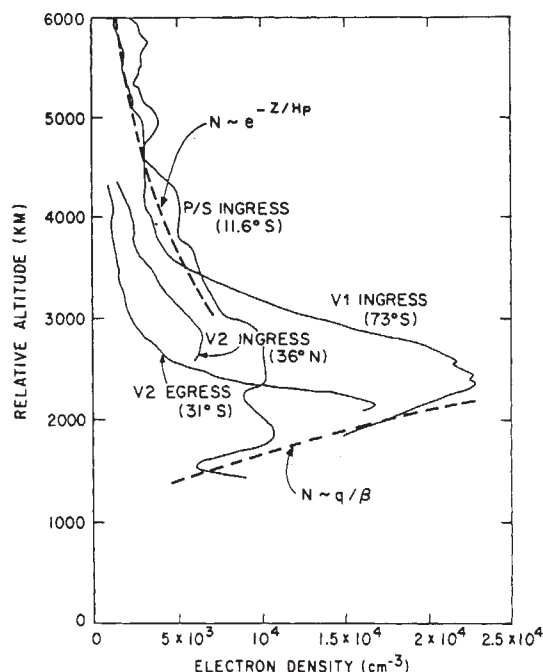


Fig. 2 Ionospheric electron density profiles deduced from Voyager 1 and 2 and Pioneer radio science data, adapted from Atreya *et al.*⁷. Altitude is referenced to the 1 bar pressure level.

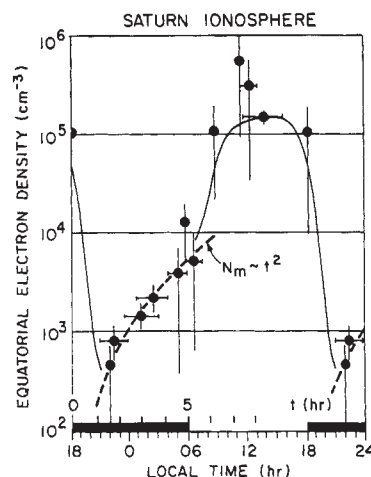


Fig. 3 Diurnal variation of maximum ionospheric electron density inferred from Voyager observations of lightning-generated radio emissions in Saturn's atmosphere, adapted from Kaiser *et al.*¹². The dashed line indicates a model fit to the nighttime increase of maximum ionospheric electron density.

and chemical loss at the homopause, the density of water throughout the (isothermal) region of interest above 1,600 km altitude is just $N_w = (FH/8D_0) \exp(-Z/H)$. Since the diffusion coefficient $D = D_0/N$, where N is the atmospheric density, the downward flux of water results in a water density proportional to the density of the H_2 atmosphere through which it flows under the condition of constant flux, that is, $N_w/N = \text{constant} = FH/8N_0D_0$.

Under these conditions a classical 'Bradbury layer', analogous to the Earth's F2 layer can form at an altitude where the chemical τ_c and diffusive τ_d time constants are comparable^{20,21}. For such a layer, the ionospheric peak forms above the level of maximum ion production owing to the upward diffusion of ions. Below that altitude the increasing chemical loss (charge transfer with water) effectively removes the major ion, so that most of the ionization anticipated by previous models is lost. The loss coefficient is $\beta = k[H_2O]$ where k is the rate coefficient of reaction B1 in Table 1. The required water concentration at the altitude of the ionospheric peak can be estimated by $N_m \equiv q(h_m)/\beta(h_m)$ where N_m is the maximum electron density at the altitudes (h_m) of the ionospheric peak, and the ion production function q and loss β are evaluated at the altitude of the peak. Taking $h_m \sim 2,500$ km, $q \sim 1 \text{ cm}^{-3} \text{ s}^{-1}$ and $N_m \sim 10^5 \text{ cm}^{-3}$, appropriate to the equatorial ionosphere at noon, yields a water concentration of $\sim 1,250 \text{ cm}^{-3}$ at 2,500 km altitude. This requires a $4 \times 10^7 \text{ cm}^{-2} \text{ s}^{-1}$ downward flux of water molecules. The maximum densities observed at the dawn and dusk terminators where the extreme ultraviolet production rate is ~ 0.1 of the maximum are ~ 1 order of magnitude lower than the noon density, as expected. Equating the chemical and diffusive time constants ($1/\beta \sim H^2/D$) yields an expected peak altitude between 2,000 and 2,500 km.

The variation of electron density with altitude can also be understood using simple approximations to 'Bradbury layer' behaviour that have been widely used in the study of the Earth's F2 layer^{20,21}. The ion distribution well above the peak is controlled by ambipolar diffusion, so $N \sim \exp(-Z/H_p)$ where H_p is the plasma scale height. This distribution is indicated in Fig. 2 assuming 800 K electron and ion temperatures, for which $H_p = 4H$. The H^+ ion density distribution below the peak is controlled by chemical loss, with $N(h) \sim q/\beta(h)$. The ion production, q , varies slowly, so, to first approximation, we expect $N(h)$ below the peak to increase with height as $\exp(h/H)$, as is illustrated in Fig. 2. This reflects the increased loss at greater depths due to greater water concentration. The peak has a width of a few scale heights and occurs at an altitude (2,500 km) where the diffusion time constant is a few hours, as does the Earth's F2 layer. However, the rapid nighttime decay of the equatorial ionospheric density, evidenced in Fig. 3, requires a more rapid loss, perhaps a downward drift related to the ionosphere-protonosphere exchange described below.

Figure 3 shows an ionospheric density minimum just after dusk and a steady increase in near-equatorial ionospheric density throughout the night. We attribute this phenomenon to a diurnal ionosphere-protonosphere exchange similar to that proposed for the Earth's nighttime F2 layer²⁰. During daylight hours, photoproduction in Saturn's ionosphere pumps protons outward along field lines to great heights. At night, a relaxation back towards the ionosphere occurs to replenish the nighttime ionospheric density. If we assume a uniform density (n) column of protons at rest at dusk, under constant downward acceleration (a) the proton influx $\Phi_p = nat$ increases linearly with time. Supposing the nighttime maximum ionospheric density occurs at high altitudes where loss is negligible, the increase in the maximum density with time is related to the influx by $N_m(t) = \int \Phi_p/H dt = (na/2H)t^2$. This increase is indicated in Fig. 3 by the dashed line, which fits the nighttime observations well with $na/2H \sim 2 \times 10^{-5} \text{ cm}^{-3} \text{ s}^{-2}$, corresponding to a proton influx increasing to a maximum of $\sim 2 \times 10^7 \text{ cm}^{-2} \text{ s}^{-1}$ just before dawn. The equivalent ion production rate at dawn due to this exchange ($\sim 0.2 \text{ cm}^{-3} \text{ s}^{-1}$) is comparable to or greater than the extreme ultraviolet production. Thus, we envision a very dynamic ionos-

phere, characterized by ion production and transport into a vast protonosphere reservoir by day, coupled with relaxation and return by night.

The increased ion losses at latitudes connected magnetically to the inner edge of Saturn's B-ring¹² can be explained by an increased local water influx of as much as 2×10^9 molecules $\text{cm}^{-2} \text{ s}^{-1}$. The erosional process described by Northrop and Hill¹³ provides a mechanism whereby sub-micrometre charged particles (agglomeration of water molecules), unstable in Saturn's ring plane, precipitate along magnetic field lines into the ionosphere. If these particles are deposited directly at the level of the ionospheric peak ($\sim 2,500$ km) the required flux is less than $2 \times 10^9 \text{ cm}^{-2} \text{ s}^{-1}$. At this maximum flux, the ring mass loss is $\sim 6 \times 10^{-14} \text{ g cm}^{-2} \text{ s}^{-1}$. The large dayside extinction was observed at latitudes of -37 to -39° (ref. 12), the maximum southerly extent of the Voyager 1 spacecraft. Averaged over $\sim 4^\circ$ latitude extent of the presumed source region in the rings ($1.52 - 1.62 R_S$), the total ring mass loss is $\sim 1.2 \times 10^6 \text{ g s}^{-1}$ or $1.6 \times 10^{12} \text{ g yr}^{-1}$, comparable to the estimated total mass influx from interplanetary meteoroids²². At this rate, 60 g cm^{-2} of material can be removed from the source region in $\sim 30 \times 10^6$ years, suggesting that this process may be responsible for the large decrease in optical depth of the rings inward of $1.62 R_S$.

The Pioneer and Voyager observations of Saturn's ionosphere and the inferences drawn from studies of Saturn lightning can be largely understood on the basis of a new model of Saturn's ionosphere in which water plays a major role. The planetwide influx of water (4×10^7 molecules $\text{cm}^{-2} \text{ s}^{-1}$) required is approximately an order of magnitude greater than that expected from photosputtering of the rings²³. Morfill *et al.*²² estimate that micrometeorite impacts on Saturn's icy rings produce between 0.75 and $6 \times 10^6 \text{ g s}^{-1}$ of vapour, $\sim 10\%$ of which is deposited in Saturn's atmosphere. This results in an estimated influx of between 0.6 and 4.5×10^7 molecules $\text{cm}^{-2} \text{ s}^{-1}$. In spite of the uncertainties in this estimate²², micrometeorite bombardment of Saturn's rings is an attractive source mechanism because it provides an explanation of the vast differences between Jupiter's and Saturn's ionospheres. An atmospheric influx of water has been previously suggested to explain features of ultraviolet spectra of Saturn²⁴ and Voyager infrared spectra of Titan²⁵. The increased influx of water at latitudes magnetically connected to the inner edge of the B-ring is not inconsistent with expected erosion rates. Finally, we infer a diurnal ionosphere-protonosphere exchange in which protons pumped into the protonosphere by day return by night.

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Production of ammonia-depleted surface layers on the saturnian satellites by ion sputtering

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It is generally believed that some type of plains-forming resurfacing has continued into the recent geological past on the surfaces of the saturnian satellites, particularly Enceladus¹⁻³. In conditions of tidally-driven heating, 'magma' could emerge and produce the plains especially evident on this satellite, as well as on Tethys, Dione, and Rhea. The magma favoured at present, considering the temperatures of the satellites and their likely formation, is the peritectic melt $2\text{H}_2\text{O}\cdot\text{NH}_3$ (refs 1-4). Surprisingly, however, visible and near-IR spectra have not shown any evidence of ammonia on the optical surface of any of the satellites, although absorption bands indicative of water ice are abundantly in evidence. On the basis of laboratory-derived data on the ion erosion of water and ammonia ices, we suggest that the satellite surfaces could have become enriched with water ice over a reasonable geological time period (10^6 – 10^9 yr) owing to preferential erosion of the ammonia by magnetosphere ion bombardment. This mechanism would be an alternative explanation to other processes invoked for the absence of ammonia spectral features. The concept is similar to that of Haff⁵ who has proposed that a crust of non-volatiles could eventually coat the Galilean satellites J3 and J4 (Ganymede and Callisto) through preferential water ice erosion (an 'armouring' process).

Sputtering rates for water ice expected on the four saturnian satellites Enceladus, Tethys, Dione and Rhea, using laboratory-measured H_2O ice erosion rates⁶ and Voyager-measured ion fluxes⁷ ≥ 30 keV in the vicinity of the satellites have recently been reported⁸. These rates are shown in Table 1, together with the expected surface loss of water ice in $\text{cm } 10^{-9} \text{ yr}^{-1}$. The results are given for Voyager 2 measured particle fluxes at Enceladus (the Voyager 1 trajectory did not go in to this distance) and Voyager 1 measured fluxes at the other three moons. The rates derived from the Voyager 2 fluxes are approximately a factor of 20 less than the Voyager 1 derived values, reflecting the temporal changes in the magnetosphere plasma population. The uncertainties in the laboratory numbers are $\sim 20\%$ (ref. 6). The surface loss determination takes into account the escape fractions expected from the satellites under gravitational forces, using laboratory-determined velocity spectra for the eroded molecules⁹. (For example, 80–90% of the eroded molecules are expected to escape Enceladus.) These molecules, sputtered from the satellites as well as the E-ring, could produce the oxygen torus around the planet.^{10,11} Sputtering of the satellite surfaces by lower-energy, corotating ions may increase the rates and the erosion depths given in Table 1. In contrast, a small atmosphere or magnetic field on any of the moons would exclude lower-

energy plasma ions from striking a satellite surface. Thus, the values in Table 1 are probably a conservative estimate of the surface effect.

The values of Table 1 indicate that a surface loss of water ice ranging from a few centimetres to several metres could be expected on Enceladus, depending on the composition of the ion-flux in the vicinity of the satellite. Determinations of this composition from the Voyager 2 data suggest that there is an appreciable flux of energetic oxygen in the vicinity of Dione and Enceladus⁷. Thus, the higher values of the erosion rate and the net erosion are probably more representative of the actual situation. The erosion values for Table 1 were calculated assuming isotropic ion bombardment of the satellites, because the pitch angle anisotropies are sufficiently small in this region of the magnetosphere⁷.

We have determined in the laboratory the erosion yield Y of frozen ammonia molecules (at $\sim 10\text{K}$) under bombardment by 1.5-MeV He^+ and Ne^+ ions. These values are $Y(\text{He}^+) \sim 140$ molecules per incident ion and $Y(\text{Ne}^+) \sim 2 \times 10^3$ molecules per incident ion. At this temperature in these experiments we found no evidence for the preferential removal from the surface of the hydrogen compared with the nitrogen. However, the NH_3 could be released as atoms and/or molecules other than ammonia, as has been reported in the case of frozen NH_3 bombarded by 3-keV argon ions¹² (absolute yields are not determined in this experiment). The $Y(\text{He}^+)$ on NH_3 is comparable with those we have measured from solid oxygen and solid carbon dioxide at these temperatures, and is ~ 18 times the erosion yield on water ice (~ 8).

The detailed erosion yield studies of water ice in the laboratory show that at temperatures $\leq 100\text{K}$, for fast light ions ($\geq 1 \text{ keV AMU}^{-1}$), the yield varies roughly as the square of the electronic stopping power of the incident ion⁶. The ratio of the 1.5-MeV He^+ and Ne^+ erosion yields on NH_3 also varies as roughly the square of the ratio of the stopping powers. We have previously found that the erosion of water ice by heavy ions depends more steeply than quadratically on the stopping power^{6,13}. From data on heavy ions incident on H_2O ice, we have $Y(1.5\text{-MeV O}^+) \sim 650$. The stopping power of neon and oxygen are quite similar at these energies, so that we can confidently use the laboratory values for $Y(\text{Ne}^+)$ in the following.

If we assume that the molecular species in a water ice-ammonia mixture sputter at a rate proportional to the individual sputtering yields, the equilibrium concentration of ammonia is $\sim 5\%$ for light-ion (proton) irradiation to $\sim 30\%$ for only heavy-ion (oxygen) bombardment. At the higher temperatures (but $< 100\text{K}$) expected on Enceladus, the ammonia fraction will be lower as the yield for the more volatile NH_3 is expected to increase with increasing temperature. Such temperature dependencies of the yield have been observed in the range 50–90 K for CO_2 and SO_2 (ref. 6), which have material binding energies bracketing that of NH_3 . At temperatures approaching the NH_3 sublimation temperature, more volatile species, such as N_2 and H_2 , are likely to be produced¹², as in the case of sputtering of H_2O ice⁶.

As the ion bombardment enhances diffusion to the surface of the more volatile species, the layer bombarded will be depleted in nitrogen down to the penetration depth of the incident ions (~ 0.3 – $3 \mu\text{m}$ for 10 – 10^3 keV protons and a tenth that for incident O^+). In fact, much deeper layers of ammonia depletion might be expected for several reasons. Ion erosion produces a very non-uniform surface and other geological processes (such as internal heating) and exogenic processes (such as micrometeoroid gardening) can bring fresh material to the surface. Also, any ice grains from the E-ring which might coat the surface will be depleted in ammonia by ion bombardment.

Taking the values of the erosion for ammonia ice estimated above, we find that for a pure ammonia ice surface the ammonia removal on Enceladus would be some tens of centimetres (erosion by protons) to tens of metres (erosion by oxygen ions) in 10^9 yr (Table 1). Hence, in 10^9 yr, it would not be unreasonable to expect the ammonia in a frozen magma to be eroded away,

Table 1 H_2O ice erosion

Satellite	Escape flux ($10^8 \text{ H}_2\text{O cm}^{-2} \text{ s}^{-1}$)		Surface loss ($\text{m } 10^{-9} \text{ yr}^{-1}$)	
	Protons incident	Oxygen incident	Protons incident	Oxygen incident
Enceladus*	0.01	1.0	0.01	1.0
Tethys†	0.05	4.0	0.06	4.0
Dione†	0.06	5.0	0.08	6.0
Rhea†	0.02	2.0	0.02	2.0

* Voyager 2 derived numbers.

† Voyager 1 derived numbers.

leaving the surface rich in water ice. If the resurfacing of the plains of Enceladus occurred $\sim 10^6$ yr ago, although the amount of ammonia eroded would be much less, enough modification would occur to prevent the detection of ammonia in the infrared. We also note that estimates of erosion over long time periods must take into account possibilities of variations in magnetosphere fluxes. The absence of reports of detection of ammonia in the magnetosphere does not indicate the absence of ammonia and/or a nitrogen by-product. In these regions, the cold- and hot-plasma instruments could not distinguish clearly between nitrogen and oxygen. Further, nitrogen from dissociated ammonia would be indistinguishable from a nitrogen contribution from Titan's atmosphere.

A process complementary to that discussed here can result from micrometeorite impacts on the satellite surfaces. Although not discussed in detail here (micrometeoroid bombardment is reviewed in ref. 14), note that such an impact will probably be enhanced by 'gravitational focusing' around Saturn, compared with the rates expected in the heliospheric ecliptic plane (for example, on the moons of Earth and Mars). NH_3 and H_2O have vastly different vapour pressures; thus, the vaporization and

fractionization produced by micrometeoroid impact would tend to deplete the surfaces of NH_3 preferentially.

These considerations present the intriguing possibility that, under an assumption of known magnetosphere charged-particle fluxes, an estimate can be made of the lower limit of the age of the last major satellite resurfacing. Furthermore, knowledge of the ammonia erosion rate can place an upper limit on the rate of continual resurfacing of a satellite surface, if that is a viable 'tectonic' process, in contrast to sudden major occurrences of resurfacing. This coupling of geological processes with space plasma physics processes is an exciting prospect, as for the case of Io's contribution to Jupiter's heavy ion magnetosphere torus.

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Midpoint scission of macromolecules in dilute solution in turbulent flow

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Drag reduction by polymer additives is commonly explained in terms of nearly complete extension of the macromolecules by high extensional strain rate regions of irrotational flow associated with turbulent bursting¹⁻⁵. The intermittent nature of the bursting process means that the polymer conformation cannot be measured directly by optical methods such as light scattering or birefringence. We have studied the degradation of narrow molecular weight distribution (MWD) polystyrene in a turbulent flow device which has been carefully constructed to minimize all sources of extensional flow except for that encountered in the turbulent flow itself. We report here the results of size exclusion chromatography of the degraded samples which show that chain scission occurs in a narrow distribution around the chain midpoint, indicating that the macromolecules are highly extended when they break. This represents the most convincing evidence yet for high chain elongation of polymers in a turbulent flow.

High chain elongation has been shown to occur in model laminar extensional flows by birefringence^{2,6}. It has also been shown that laminar extensional flow induces scission of polymers in a fairly narrow distribution centred on the chain midpoint^{6,7}. Midpoint scission itself is evidence of high chain extension since the highly elongated state is the only conformation of a randomly coiling macromolecule that would cause the maximum hydrodynamic force to be at the centre of the contour length of the chain.

The degradation studies were done in a single-pass gas pressure-driven flow device (see Fig. 1 legend for a description of the device). To perform a degradation run, the degraded sample collection reservoir and flow tube were filled with pure solvent and the feed solution reservoir was filled with the polymer solution. The system was pressurized to 20-87 atm and the

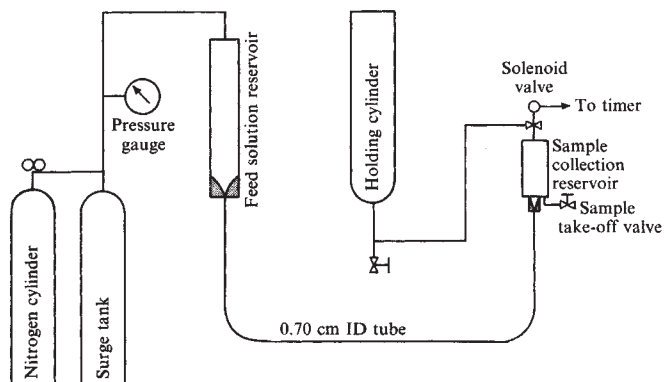


Fig. 1 Schematic diagram of the turbulent flow degradation apparatus. The apparatus comprises two reservoirs of 8.9 cm ID (internal diameter) connected by a 0.70-cm ID stainless steel tube 244 cm long. The entrance to the tube from the feed solution reservoir comprises a conical funnel machined on a 4.1 cm radius that smoothly reduces the diameter from that of the reservoir to that of the tube. (This lowered by an order of magnitude the extensional strain rates caused by the entrance, and was necessary as Culter *et al.*¹⁰ have shown that entrance effects can dominate polymer degradation even in turbulent flow.) The exit from the tube to the degraded sample collection reservoir consists of a cone of 7° angle which reduces the fluid velocity by an order of magnitude. A timer-controlled solenoid valve is mounted downstream of the degraded sample collection reservoir, and solution leaving the solenoid valve is sent to a holding cylinder. A sampling valve is mounted on the degraded sample collection reservoir upstream of the solenoid valve.

solenoid valve was opened for 1.5-4 s. Polymer solution passed through the degraded sample collection reservoir and solenoid valve to the holding cylinder. While the concentration of the polymer solution changed in the degraded sample collection reservoir during the experiment, the MWD was always that of the polymer that had experienced the turbulent flow.

The molecular weight distributions of the samples were determined using size exclusion chromatography (SEC). The system consisted of a pair of Waters Ultrastaygel (10^5 Å and 10^6 Å

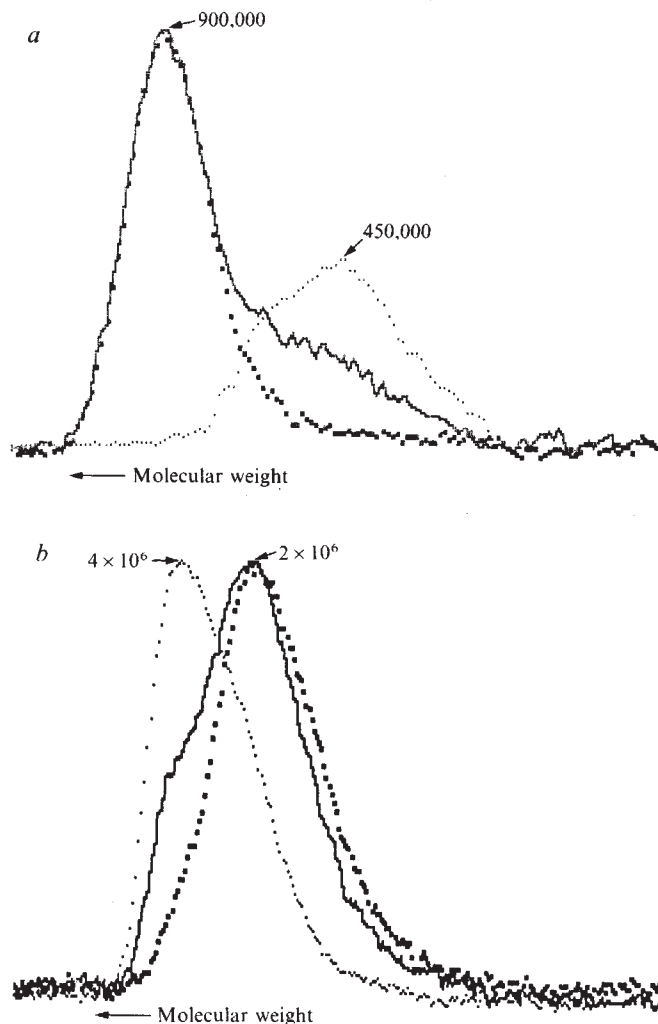


Fig. 2 *a*, Chromatograms of original (---) and degraded (—) 900,000-MW polystyrene., 2.5 times the difference (degraded—original). *b*, Chromatograms of original (.....) and degraded 3.84×10^6 -MW polystyrene. —, Degraded sample (single pass at 21 atm total pressure drop); ---, degraded sample (single pass at 40 atm total pressure).

pore size) columns; the plate count for the system was greater than 30,000 plates. Anionically polymerized polystyrene of molecular weight (MW) 900,000 (ratio for weight average MW to number average = 1.04 by SEC) was obtained from Pressure Chemical Co. and a sample of MW 3.84×10^6 was purchased from Toyo Soda Ltd. The 3.84×10^6 -MW sample had a dispersity of 1.15 by SEC. The degradations were done at a polymer concentration of 50 p.p.m. in chloroform. The shear viscosity of the solution was identical to that of the pure solvent.

Figure 2*a* shows chromatograms of undegraded 900,000-MW polystyrene and the same sample after a single pass through the apparatus at 87 atm total pressure drop (Reynolds number = 730,000 and the wall shear stress was estimated as $3,000 \text{ N m}^{-2}$). The height of the chromatogram has been normalized to simplify comparison. The degraded sample exhibits a shoulder centred at MW 450,000. When the chromatogram of the original sample is subtracted from that of the degraded sample (normalized according to peak area), the difference curve peaks at MW 450,000 (Fig. 2*a*). This proves that scission of the macromolecules occurs in a fairly narrow distribution around the chain midpoint. Midpoint scission is also confirmed by the data for the 3.84×10^6 -MW polymer in Fig. 2*b*. The chromatogram of the undegraded sample consists of a single peak at MW 4×10^6 ; for the sample degraded at a system pressure drop of 21 atm, there is a shoulder at MW 4×10^6 due to the unbroken fraction and a peak centred at MW 2×10^6 due to the

degraded material. At 40 atm pressure drop, essentially all of the original polymer is degraded and there is a single peak centred at MW 2×10^6 (Fig. 2*b*). These data are consistent with midpoint scission governed by a rate constant which decreases with decreasing molecular weight.

By using simultaneous birefringence and degradation measurements in a cross-slot flow device, Odell *et al.*⁶ have shown that midpoint scission occurs when the macromolecule is fully extended in an extensional flow. Recent SEC degradation studies done in our laboratory with a sudden contraction device confirm midpoint scission of polymer chains in extensional flow. As discussed in ref. 7, we model the extended polymer chain as a string of beads aligned with the flow to calculate the drag force on the chain. Following Zhurkov⁸, main-chain bond scission of a bond in tension is modelled as a first-order kinetic process with the rate constant given by

$$k_i = A \exp [-(E_a - F_i \Delta r)/kT] \quad (1)$$

where k_i is the first-order rate constant for scission of the i th bond, E_a is the bond energy, F_i is the force on that bond, Δr is the distance a bond must be separated to break, and k is Boltzmann's constant. Using this model, drag forces obtained from curve fitting of experimental data are of the correct order of magnitude. The width of the second peak is correctly predicted by equation (1). These results, taken in conjunction with the data of Odell *et al.*⁶, provide convincing evidence that full chain extension does indeed occur in extensional flow before chain scission and that this leads to scission centred on the chain midpoint.

Thus, the data presented here, showing midpoint scission of macromolecules in turbulent flow, strongly imply that the polymer chains can indeed be fully extended by turbulent flow, as well as by laminar extensional flow. Previous studies have suggested that scission is centred on the midpoint⁹; this notion was based primarily on the observation that the molecular weight distributions of broad MWD polymers are narrowed by degradation. The chromatograms of the degraded narrow MWD samples presented here provide the most direct evidence that we are aware of for high extension of randomly coiling macromolecules in turbulent flow.

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Ocean-atmosphere coupling over monsoon regions

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In monsoon regions, the seasonal migration of the intertropical convergence zone (ITCZ) is manifested as a seasonal reversal of winds. Most of the summer monsoon rainfall over India occurs owing to synoptic and large-scale convection associated with the continental ITCZ (Fig. 1). We have investigated the interaction between these large-scale convective systems and the ocean over which they are generated¹⁻³, concentrating on the relationship between organized convection over the Indian Ocean and sea surface temperature (SST). We report here that on a monthly basis

the degree of cloudiness correlates well with SST for the relatively colder oceans, but when SST is maintained above 28 °C it ceases to be an important factor in determining the variability of cloudiness. Over the major regions of convection east of 70° E, which are warm year after year, the observed cloudiness cannot be correlated with variations in SST.

Organized convection over the equatorial Indian Ocean has an important role in maintaining the continental ITCZ¹⁻³ (Fig. 2), and the degree of continental cloud cover is linked to that over the ocean. The most important factor governing generation and maintenance of synoptic and large-scale cloudiness in the tropics is SST^{4,5}. Variability of large-scale convection over the Pacific has been shown to be intimately related to variations in SST^{6,7}. However, the coupling between the Indian Ocean and the continental ITCZ is more complex, involving correlations between the SST and oceanic cloudiness on the one hand and the oceanic and continental cloudiness on the other.

Not surprisingly, studies of the relationship between monsoon rainfall over India and the SST anomalies over different parts of the Indian Ocean, Arabian Sea and Bay of Bengal have yielded rather low correlation values⁸⁻¹⁰. In fact, the results of these studies differ even in the sign of the correlations obtained (perhaps because the periods studied were different, or there were biases in the data, etc.). The results of different investigations of the relationship between SST anomalies over the Indian Ocean and rainfall over India using general circulation models are also ambiguous¹¹⁻¹³. The one unequivocal result of all quantitative numerical studies is the reduction (enhancement) in precipitation directly over a cold (warm) SST anomaly.

As a first step towards unravelling the complex interaction between the continental ITCZ and the SST over monsoon regions, we investigated the relationship of the synoptic and large-scale convective cloudiness over the Indian Ocean to SST during the summer monsoon.

The basic data available for 1966-72 are: (1) daily values of a cloudiness index (ranging from 1 to 9) at each 2.5° latitude-longitude square over the tropics, based on operational nephelanalysis prepared by the National Environmental Satellite Service of NOAA and compiled by Sadler and associates^{14,15}, and henceforth called the Sadler index; and (2) monthly SST over the oceanic regions between 40° E and 100° E and north of 10° S for 5° latitude-longitude squares compiled by Joseph and Pillai¹⁰ from the data collected by the voluntary observing fleet of over 40 maritime nations and stored at the National Data Centre of the India Meteorological Department.

A comparison of the daily distribution of the Sadler index with the imagery of the hemispheric mosaics analysed previously in an investigation of the variation of the ITCZ over Indian longitudes², indicated that synoptic and large-scale convection (for example, ITCZ) is associated with values of ≥ 6 of the Sadler index. Thus, we have concentrated here on epochs characterized by Sadler index ≥ 6 and define the cloudiness intensity at any grid point for any month as the sum of the Sadler index for the days on which it is ≥ 6 .

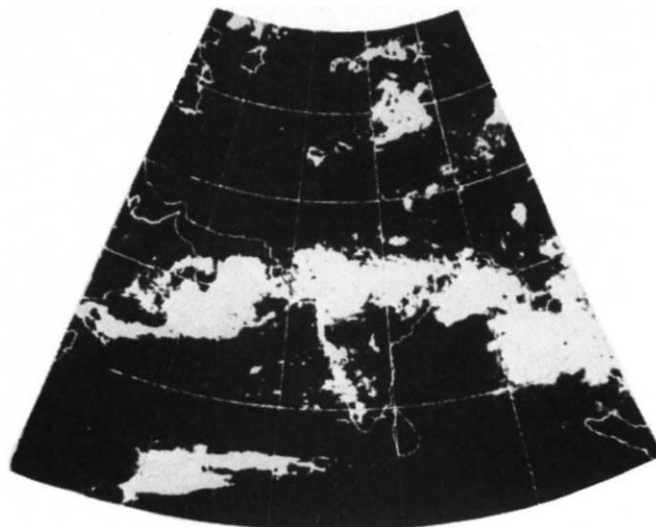


Fig. 1 The ITCZ over Indian longitudes on 8 July 1973 (after ref. 1).

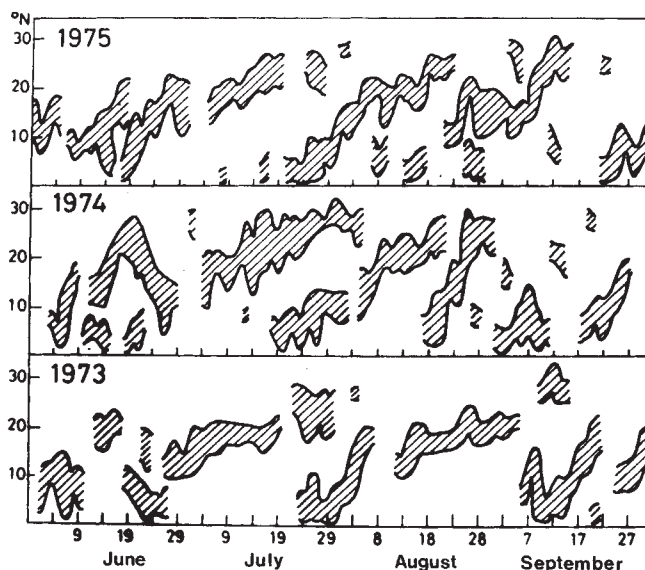


Fig. 2 Variation of the location and latitudinal extent of the ITCZ at 90° E during the monsoon seasons of 1973-75.

For a 5° grid, there are 58 squares (cells) in our study region for which SST and cloudiness intensity are given for each month (June-September) of 1966-1972. This corresponds to over 1,600 cell-months for the seven monsoon seasons. The distribution of these cell-months in the SST cloudiness intensity plane is shown in Fig. 3. The most remarkable feature of this distribution is the restriction of the points to the warmer side of the lines *ab*, *bc*. A given level of cloudiness intensity occurs only if the SST is higher than a specific value, which is clearly a necessary condition for occurrence of organized convection. For SST below 28 °C there is a well defined value for the maximum cloudiness intensity (which increases linearly with SST), whereas above 28 °C it seems to become independent of SST, although there are too few points with large cloudiness intensities to make definitive statements about this behaviour. Up to about 28 °C, the minimum cloudiness intensity is zero (that is, a Sadler index of < 6 for each day of the month) but above 28 °C it increases with SST. The probability of occurrence of different cloudiness intensities for a given SST also shows a marked change across 28 °C (Fig. 4). Whereas the most probable intensity is zero below 28 °C, it abruptly shifts to ~ 40 units above this SST. These results suggest that a SST of 28 °C is the threshold for active generation of organized convection.

Table 1 Correlation between SST and degree of cloudiness over Indian Ocean

SST greater than (°C)	No. of data points	Correlation coefficient ($\pm 95\%$ confidence limits)
24.5	1,602	0.564 ± 0.034
25.0	1,568	0.548 ± 0.035
25.5	1,502	0.517 ± 0.037
26	1,418	0.468 ± 0.041
26.5	1,340	0.418 ± 0.045
27	1,212	0.364 ± 0.050
27.5	1,064	0.282 ± 0.056
28	823	0.183 ± 0.067
28.5	468	0.094 ± 0.092
29	190	0.01

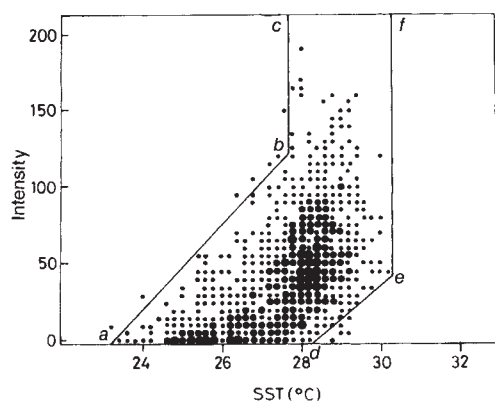


Fig. 3 Cloudiness intensity plotted against SST for June–September 1966–1972. The number of cell-months at each location is indicated: •, 1–4; ●, 5–9, ●, >10.

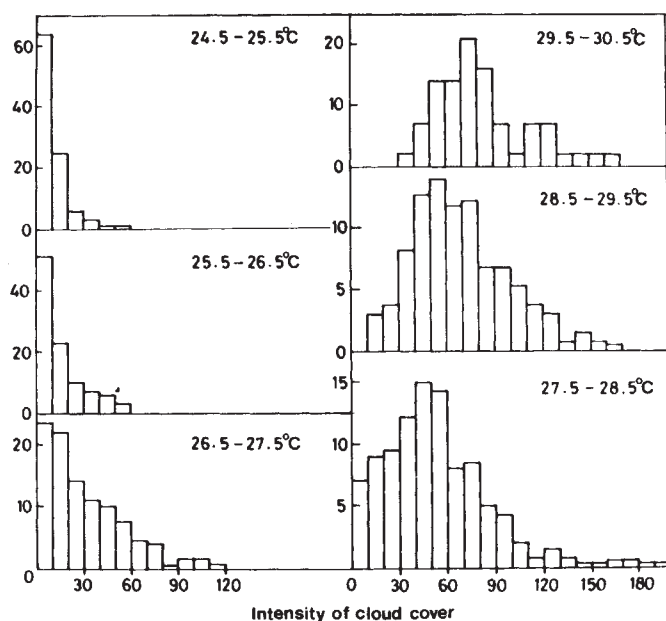


Fig. 4 The percentage of cell-months with different cloudiness intensities for SST in the given 1 °C interval.

The probability of occurrence of cloudiness of 60 units (that is, about 10 'cloudy' days per month) is zero for SST below 26.5 °C, which is the Palmen¹⁶ threshold for tropical cyclogenesis. This probability increases with SST, reaching a value of 0.65 (and not unity) over the warmest waters, which demonstrates clearly that high SST is a necessary but not sufficient condition for sustained cloudiness over a significant portion of the month.

The correlation between SST and cloudiness intensity based on the entire summer data set is large and positive: 0.566 ± 0.033 (95% confidence limits). Most of this correlation arises from restriction of the cloudy region to relatively warm waters, and once the threshold of 28 °C is crossed, the intensity of cloud cover is no longer dependent on the SST (as seen in Table 1 for the correlation coefficient for restricted data sets comprising only cells with SST above the specified limit). Of the many factors that are important for intense convection such as lower tropospheric convergence, SST, etc., SST ceases to be the critical limiting factor when maintained above the threshold. Thus, over the warm oceans¹⁷ east of 70° E, which are the major regions of organized convection, the correlation is poor (and over some parts even negative). In fact, the observed variability in cloudiness during 1966–72 over the north Indian Ocean cannot be attributed to the variation of the monthly SST.

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1,700-Myr greenstone volcanic successions in southwestern North America and isotopic evolution of Proterozoic mantle

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The formation of continental crust over the past 3,800 Myr has depleted the mantle of large-ion lithophile elements¹. A knowledge of the timing of the depletion and the degree of chemical homogeneity of the depleted mantle over the history of the Earth is important to understand the development of the continents, their recycling back into the mantle^{2–6}, and the nature of convection and mixing in the ancient mantle. This may be inferred by determining the initial ¹⁴³Nd/¹⁴⁴Nd, ¹⁷⁶Hf/¹⁷⁷Hf, and ⁸⁷Sr/⁸⁶Sr ratios of igneous rocks that were derived from the mantle at various times. Initial ¹⁴³Nd/¹⁴⁴Nd ratios of ~1,700-Myr greenstones from the Rocky Mountains range from $\epsilon_{Nd} = +3.3$ to $+6.5$, the highest values yet observed for mid-Proterozoic rocks. These data constrain the change in ϵ_{Nd} of depleted mantle to be only 2–3 ϵ units over the past ~1,700 Myr which supports the hypothesis of significant recycling of continental crust into the mantle^{2,3}.

Nd and Sr isotopic data were collected from ~1,700-Myr old metavolcanic rocks along a 750-km long traverse from near the Wyoming Archaean province to central New Mexico (Fig. 1). These predominantly mafic oceanic volcanic rocks probably represent primitive island-arc type magmatic effusions which are believed to be the raw materials for the generation of continental crust⁷. Lack of evidence for crust older than 1,800 Myr (ref. 8), and trace element data^{8–10} suggest that the samples are not significantly contaminated by older crust. The objectives of analysing this suite are to determine the Nd and Sr isotopic composition of the mantle 1,700 Myr ago, and to assess the meaning of the isotopic data for the evolution of the upper mantle and for the petrogenesis of these and other rocks of similar ages^{11,12}.

The volcanic greenstone successions sampled are summarized in Table 1. The U–Pb (zircon) crystallization ages for these rocks increase from south to north towards the Archaean craton (Fig. 1) (refs 13–15, 18 and S. Bowring, in preparation). The samples from the Green Mountain Formation were collected from within 3 km south of the Wyoming Shear Zone^{17–18}, which separates the $\leq 1,800$ Myr-old crust from the $> 2,500$ Myr Archaean province to the North.

The analytical methods are described elsewhere^{19,20}, except that neodymium isotopic measurements were made using an NdO⁺ ion beam rather than Nd⁺. Dissolution procedures are detailed in Table 1.

The Sm–Nd and Rb–Sr isotopic data are listed in Table 1. The initial ϵ_{Nd} and ϵ_{Sr} values of the samples have been calculated

Table 1 Rb-Sr and Sm-Nd isotopic data on greenstone belts, southwestern USA

Sample	Description	Sr(p.p.m.)	⁸⁷ Rb/ ⁸⁶ Sr	⁸⁷ Sr/ ⁸⁶ Sr _m [†]	ε _{Sr} (T) [‡]	Nd(p.p.m.)	¹⁴⁷ Sm/ ¹⁴⁴ Nd	¹⁴³ Nd/ ¹⁴⁴ Nd _m [†]	ε _{Nd} (T) [*]
Tijeras greenstone ³⁴ (1,680 Myr)									
TJ-1	Tholeiite	102.7	0.5261	0.70639 ± 2	-126	4.556	0.2275	0.512463 ± 20	5.6
MZ-2	Tholeiite	137.9	0.1772	0.70402 ± 3	-39.3	4.960	0.2295	0.512525 ± 20	6.4
MZ-3	Tholeiite	132.7	0.6803	0.70855 ± 4	-148	5.893	0.2224	0.512428 ± 18	6.0
MZ-5	Tholeiite	138.4	0.0307	0.70373 ± 5	+6.9	5.234	0.2234	0.512410 ± 20	5.5
MZ-6	Tholeiite	60.40	0.0245	0.70369 ± 3	+8.2	4.490	0.2364	0.512521 ± 16	4.8
Pecos greenstone ³⁵ (1,720 Myr)									
79-12-26	High-Mg tholeiite	289.8	0.2378	0.70875 ± 3	+6.1	2.078	0.1618	0.511642 ± 15	3.9
79-22-26	High-Mg tholeiite	216.1	0.3838	0.71196 ± 3	+0.6	1.745	0.1725	0.511756 ± 19	3.8
79-18-26	Ultramafic	4.285	0.2468	0.71100 ± 4	+35.0	0.7852	0.1053	0.511154 ± 20	6.9
		4.364	0.2475	0.71096 ± 6	+34.2	0.8113	0.1056	0.511145 ± 19	6.7
79-23-26	Ultramafic	19.37	0.0444	0.70557 ± 3	+28.7	1.074	0.1364	0.511391 ± 14	4.6
Dubois greenstone ⁹ (Cachetopa Creek) (1,740 Myr)									
B-6	Tholeiite	304.5	0.0559	0.70388 ± 3	+0.7	12.69	0.1843	0.511942 ± 25	4.9
B-8	Tholeiite	226.6	0.2335	0.70750 ± 3	-11.0	11.06	0.1876	0.511995 ± 16	5.2
A-6	Tholeiite	394.0	0.0207	0.70295 ± 4	+0.1	20.69	0.1372	0.511407 ± 25	4.9
B-11	Tholeiite	464.6	0.2166	0.70772 ± 4	-1.9	27.44	0.1450	0.511476 ± 21	4.6
SAM§	Rhyolite	196.3	1.051	0.72819 ± 3	-7.6	40.82	0.1217	0.511210 ± 20	4.8
		196.4	1.057	0.72818 ± 4	-9.8	41.00	0.1217	0.511240 ± 21	5.2
Green Mountain Formation ¹⁶ (1,780 Myr)									
GM09	Tholeiite	539.1	0.0118	0.70293 ± 4	+7.1	14.50	0.1337	0.511287 ± 21	3.7
GM16	Tholeiite	464.6	0.0546	0.70411 ± 3	+4.7	16.89	0.1330	0.511308 ± 22	4.3
GM33	Tholeiite	497.3	0.0611	0.70431 ± 3	-2.7	21.47	0.1324	0.511258 ± 36	3.4
GM51	Tholeiite	606.3	0.1087	0.70605 ± 3	+12.6	19.77	0.1322	0.511266 ± 24	3.6
GM37§	Dacite	277.0	0.7297	0.72976 ± 3	+124	33.81	0.1156	0.511073 ± 21	3.7
GM27§	Rhyolite	172.8	0.6921	0.71923 ± 2	-12.4	45.64	0.1126	0.511041 ± 16	3.7
		173.0	0.6994	0.71944 ± 3	-12.1	45.68	0.1127	0.511063 ± 25	4.1
GM67§	Rhyolite	132.5	1.342	0.73652 ± 5	-3.1	30.18	0.1091	0.510995 ± 17	3.5
		134.1	1.344	0.73658 ± 3	-2.9	30.90	0.1088	0.511014 ± 26	4.1

* Calculated with respect to a chondritic reservoir with composition today of ¹⁴³Nd/¹⁴⁴Nd = 0.511836, ¹⁴⁷Sm/¹⁴⁴Nd = 0.1967, λ_{Sm} = 0.00654 × 10⁻³ Myr⁻¹. Error for calculated initial Nd ratio is ±0.5 ε units. The initial ¹⁴³Nd/¹⁴⁴Nd and ⁸⁷Sr/⁸⁶Sr ratios have been converted to ε_{Nd} and ε_{Sr} values, which indicate how the ratios differ from those expected for primitive mantle reservoirs at the time of formation of the lavas^{28,36}.

$$\epsilon_{Nd} = 10^4 \left[\frac{{}^{143}\text{Nd}/{}^{144}\text{Nd}_{\text{rock}}}{{}^{143}\text{Nd}/{}^{144}\text{Nd}_{\text{CHUR}}} - 1 \right]$$

† Uncertainties at the 95% confidence level. ¹⁴³Nd/¹⁴⁴Nd normalized to ¹⁴⁶Nd/¹⁴²Nd = 0.636151. ⁸⁷Sr/⁸⁶Sr normalized to ⁸⁶Sr/⁸⁸Sr = 0.1194.

‡ Calculated with respect to a uniform reservoir with present composition of ⁸⁷Sr/⁸⁶Sr = 0.7045, ⁸⁷Rb/⁸⁶Sr = 0.0827, λ_{Sr} = 0.0142 × 10⁻³ Myr⁻¹. Error for calculated initial Sr ratio is ±0.5 ε units for low Rb/Sr to ±0.9 for high Rb/Sr.

§ These rhyolites were spiked and then dissolved in sealed Teflon bombs at 180 °C for 7 days. A second aliquot of each rhyolite was also completely dissolved in Teflon beakers for comparison (second entry). The small differences observed are within the range expected for sample heterogeneity (for example, see two aliquots of 79-18-26) and the calculated initial isotopic ratios are the same within analytical uncertainty.

using the zircon U-Pb ages (Table 1, Figs 2, 3). All of the initial ε_{Nd} values are positive. The Green Mountain Formation (GMF) has values of +3 to +4, which are comparable with those determined previously for the Twilight Gneiss and the Idaho Springs Formation orthogneiss¹¹. The ε_{Nd} values for the Dubois and Tijeras greenstones are in the range +4.5 to +6.5, which are the highest values yet measured on any rocks of this general age^{21,22} (Fig. 2). The basaltic rocks of the Pecos greenstones have ε_{Nd} values that are similar to those of the GMF. One ultramafic sample (79-18-26), however, has ε_{Nd} as high as +6.9, although this sample could be especially susceptible to post-crystallization disturbance because of its low Nd content. A second ultramafic sample (79-23-26) collected only 300 m from the first has a calculated initial ε_{Nd} of +4.5, which is more consistent with values measured for the Pecos basalts.

Because small amounts of crustal contamination would cause the ε_{Nd} values of magmas to be lowered, we infer that the highest ε_{Nd} values measured are most likely to be representative of the upper-mantle. The other rock suites may be affected by minor involvement of preexisting Archaean crust, which would have had an average ε_{Nd} value of about -10 (Fig. 4). The samples with the lowest ε_{Nd} values occur closest to the Archaean craton, which is qualitatively consistent with the contamination hypothesis. However, a wide variety of rock types from a fairly broad area all seem to have been affected to the same extent (GMF, Pecos, Idaho Springs Formation and the Twilight Gneiss). This implies that there was a rather effective and large-scale process of crust-mantle mixing involved. Only a small proportion of crust is necessary to explain the shift from ε_{Nd} = +6 to ε_{Nd} = +4 (1-5% depending on the process), so even the most 'contaminated' rocks still largely represent primitive mantle-derived materials.

The relatively high initial ε_{Nd} values of up to +6.5 (or +6.9) have important implications for crust-mantle evolution models^{3,5,6}. The relationship between these data and data on island-arc type mafic volcanic rocks of other ages is illustrated in Fig. 2. The data suggest that the ε_{Nd} value of the depleted mantle source of continental-type materials has increased by only about 2 or 3ε units over the past 1,800 Myr. The growth of ε_{Nd} expected for *in situ* radioactive decay in the mantle is given by: Q_{Nd} f_{Sm/Nd} (ref. 3) where Q_{Nd} is 25.13 × 10⁻³ Myr⁻¹ and f_{Sm/Nd} is the Sm/Nd enrichment factor relative to chondrites. The highest f_{Sm/Nd} value measured on a 1,700 Myr greenstone (MZ-6) of +0.20 is similar to the highest values measured on young mid-ocean ridge basalts (MORB)²³. Based on partial melting models, the f_{Sm/Nd} value of the mantle source should be larger¹. Consequently, the growth rate of ε_{Nd} in the mantle due to *in situ* radioactive decay is expected to be >5 × 10⁻³ Myr⁻¹, or ~3-5 times faster than observed. These data verify the discrepancy noted previously³ and emphasize the need to have low-ε_{Nd} materials injected into the mantle, to slow the growth of the upper mantle ε_{Nd} value. We believe that this low-ε_{Nd} material is old continental crust, although other explanations have been proposed²⁴. The data also constrain the depleted mantle curve for mid-Proterozoic time to significantly higher ε_{Nd} values than previously suggested^{11,25} (Fig. 2).

Poor correlation of measured ⁸⁷Rb/⁸⁶Sr with ⁸⁷Sr/⁸⁶Sr (Table 1) indicates disturbance of the Rb-Sr systematics of the samples. This casts doubt on their reliability as estimators of the mantle ⁸⁷Sr/⁸⁶Sr value. The calculated initial values for the samples are compared with present-day oceanic basalt values on the ε_{Nd}-ε_{Sr} diagram^{30,31} (Fig. 3). This representation assumes that the present-day whole Earth ⁸⁷Sr/⁸⁶Sr and ⁸⁷Rb/⁸⁶Sr ratios are 0.7045 and 0.0827 respectively²⁶. The ε_{Sr} values for the Proterozoic

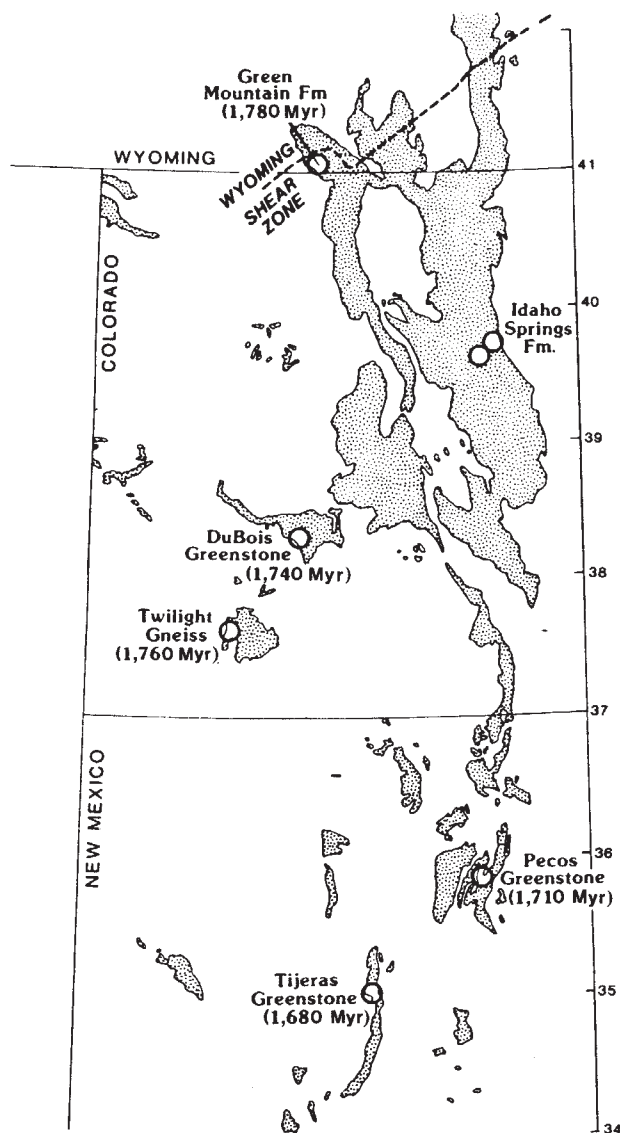


Fig. 1 Precambrian outcrop map of Wyoming, Colorado and New Mexico, USA showing sample locations and U-Pb ages from zircons (adapted from ref. 8).

metabasalts with $^{87}\text{Rb}/^{86}\text{Sr} < 0.1$ appear to be too high relative to their ϵ_{Nd} values when compared with young oceanic lavas, especially those from mid-ocean ridges^{23,26,27}. All except three of the samples plot to the right of the line $\epsilon_{\text{Sr}} = -2.7\epsilon_{\text{Nd}}$. The three anomalous samples have impossibly low calculated initial ϵ_{Sr} values and are clearly disturbed. Three of the samples with higher $^{87}\text{Rb}/^{86}\text{Sr}$ ratios lie close to the line. The apparent shift of ϵ_{Sr} values to the right on the diagram is similar to altered young oceanic basalts and some island-arc lavas²⁶⁻²⁹. This, and the relatively large range of observed ϵ_{Sr} values lead us to conclude that the samples do not precisely define the ϵ_{Sr} value of the mantle sources of the lavas. Most simple models of mantle isotopic evolution predict that the ϵ_{Sr} value of depleted mantle reservoirs should lie near the MORB-whole Earth line for all time. This follows from the observation that the partitioning of Sr and Nd between the mantle and the continental crust may be similar³⁰, though this has not been proven. ϵ_{Nd} values of +6 should correspond to $\epsilon_{\text{Sr}} = -16$ or $^{87}\text{Sr}/^{86}\text{Sr} \approx 0.7014$ at $\sim 1,700$ Myr ago. Most previous studies of mid-Proterozoic rocks have failed to find initial $^{87}\text{Sr}/^{86}\text{Sr}$ values this low^{21,31}. Our data are consistent with this low value for the mantle, but cannot be considered proof because of the uncertainties associated with

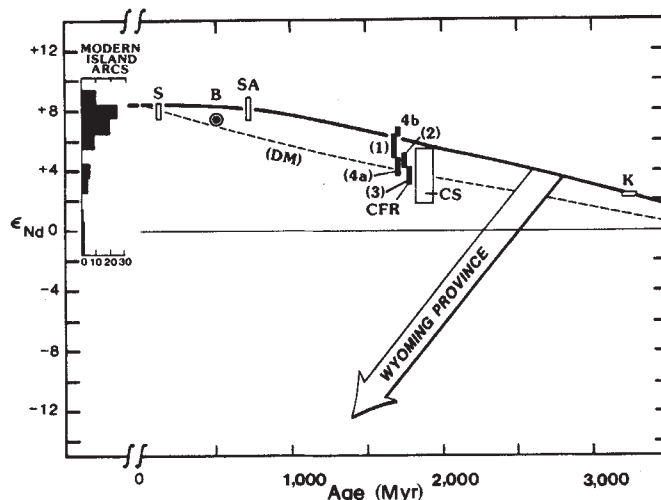


Fig. 2 Nd isotopic evolution diagram for the depleted mantle. Data from this work are shown in solid rectangles. (1), Tijeras; (2), Dubois; (3), Green Mountain Formation, which corresponds to the range for the Colorado Front Range¹¹ (CFR), (4a) Pecos, (4b) possibly altered ultramafic sample from Pecos. Large arrow indicates average evolution for Archaean crust^{37,38}. Other isotopic data: K, Kambalda³⁹; CS, Cape Smith²¹; SA, Saudi Arabia⁴⁰; B, Bay of Islands ophiolite²⁹; S, Samail ophiolite⁴¹. A histogram of published ϵ_{Nd} values for modern intra-oceanic island arcs apparently free from contamination by older crust is shown at left^{28,42-50}. All samples with $\epsilon_{\text{Nd}} < 6.5$ are either boninites or from the Lesser Antilles. Dashed curve (labelled DM) is isotopic evolution of depleted mantle proposed by DePaolo¹¹ and Allègre and Rousseau²⁵.

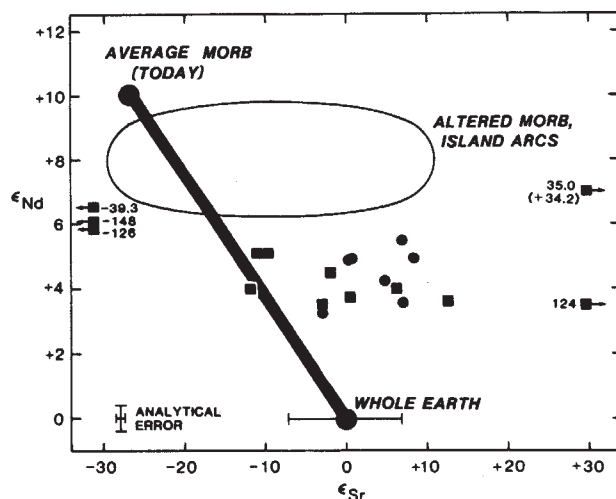


Fig. 3 ϵ_{Nd} versus ϵ_{Sr} diagram, showing initial ratios calculated for samples with $^{87}\text{Rb}/^{86}\text{Sr} < 0.1$ (●) and $^{87}\text{Rb}/^{86}\text{Sr} > 0.1$ (■). (Data that plot outside range of graph are indicated by arrows with ϵ_{Sr} values.) Oceanic correlation line for modern basalts corresponds to $\epsilon_{\text{Sr}} = -2.7\epsilon_{\text{Nd}}$. Value and estimated errors for bulk Earth isotopic composition are from ref. 26. Depleted mantle should always have values along the correlation line (see text). Three samples plot near this line, but typically the Rb-Sr systems have been strongly disturbed by later metamorphic or weathering events.

the effects of metamorphism. We emphasize that the observed initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the high-Rb/Sr rocks (as low as 0.7023) would normally be considered to be quite low, but the correct mantle value is probably lower.

Greenstones of 1,680–1,780 Myr age in the southern Rocky Mountains have high ϵ_{Nd} values and high Sm/Nd ratios that indicate the existence of relatively homogenous and widespread depleted mantle reservoirs similar to those sampled today in oceanic magmatic arcs. The data strengthen the observation³

that the growth of ϵ_{Nd} in the upper mantle has been retarded over the past 2,000 Myr, probably because of recycling of continental crust. The general tendency for samples nearer the Archaean craton to have lower initial ϵ_{Nd} values argues for contamination with small amounts of Archaean crust. Yet these samples—the GMF, Twilight Gneiss and Idaho Springs Formation—all have the same calculated initial $\epsilon_{Nd} = 3.7 \pm 0.5$, which implies vigorous and thorough regional mixing of a small amount of crust into the mantle near the Archaean plate boundary. The almost ubiquitous disturbance of the Rb–Sr system in crustal rocks makes it difficult to determine a precise $^{87}Sr/^{86}Sr$ evolution curve for depleted mantle. Continuing efforts to establish this curve are, however, extremely important in the context of establishing the history of crust–mantle evolution.

The Nd data confirm earlier conclusions that large segments of the crust in southwestern North America were derived from the mantle ~1,800 Myr ago³². Compared with the Mesozoic and Cenozoic patterns, this suggests major contrasts in rates, and possibly styles, of plate tectonics in middle Proterozoic time³³.

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A mechanism for magmatic accretion under spreading centres

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The most active volcanic zone on Earth occurs along the global mid-ocean ridge system where the lithosphere is pulled apart and new oceanic crust is created at rates between 10 and 200 km Myr⁻¹. This volcanism is segmented, and is concentrated along the axial valleys and highs of slow- and fast-spreading ridges respectively, with transform fault zones separating, and generally offsetting, each active ridge segment. Volcanic activity may be nearly absent at the ridge transform intersection where a slow-spreading ridge is offset, and greatly reduced where a fast-spreading ridge is offset, implying thinner crust in these regions¹. The source of magmas erupted along ocean ridges is the underlying mantle, which undergoes decompression melting when rising to fill the gap between the spreading plates. The resulting magma aggregates in the upper mantle and ascends to the crust due to its low density compared with the parent mantle rock. The melt is believed to pool in crustal magma chambers—whence it periodically erupts to the surface. Typically, the formation, ascent and aggregation of magmas along the mid-ocean ridges is regarded two-dimensionally and modelled in a section through the crust and mantle across the strike of the ridges (see ref. 2). Here, however, we consider the problem with a three-dimensional model for which we provide supporting geological evidence, in which the magmas rise as a result of a gravitational instability (modified Rayleigh–Taylor) in the underlying partial melt zone.

It is reasonable, to a first approximation, to assume that the mantle is behaving as a viscous newtonian fluid, so we can expect the upwelling to be two dimensional with a maximum under the smoothed location of the spreading centre. If this is the case, we also postulate that a linear region of high-melt content exists in the mantle below the ridge where melt aggregates from the rising asthenosphere. This region could be approximated as a horizontal cylindrical body with relatively low viscosity and density compared with the overlying mantle. In such circumstances, it will develop a gravitational instability leading to regularly spaced vertical protrusions. We have conducted some simple experiments in which a water–glycerine mixture was quickly injected into glycerine along a horizontal line. Although this line will gradually rise because the water–glycerine mixture is less dense than the pure glycerine, an instability will always also develop as shown in Fig. 1 and lead to semi-spherical pockets. It is reasonable to expect that a linear

Table 1 Composition of postulated parental mantle, abyssal peridotites and a primitive abyssal basalt

	Parental mantle models			Fracture zone peridotites§	Primitive abyssal basalt tholeiite
	Pyrolite*	Pyrolite†	Xenoliths‡		
SiO ₂	46.1	45.0	42.9	43.6	50.9
TiO ₂	0.02	0.17	0.2	0.02	0.89
Al ₂ O ₃	4.3	4.4	5.8	1.18	16.0
FeO	8.2	7.6	9.2	8.20	8.83
MgO	37.6	38.8	37.2	45.2	9.02
CaO	3.1	3.4	3.7	1.13	12.4
Na ₂ O	0.04	0.40	0.7	0.02	2.08
Cr ₂ O ₃	—	0.45	0.2	0.22	—
NiO	—	0.26	0.2	0.22	—
Mg/(Mg + Fe)	0.891	0.901	0.878	0.908	—

* Pyrolite model²¹.

† Pyrolite model²².

‡ Upper mantle model²³.

§ Average abyssal peridotite⁹.

|| Primitive abyssal tholeiite (TR154–1802)²⁴.

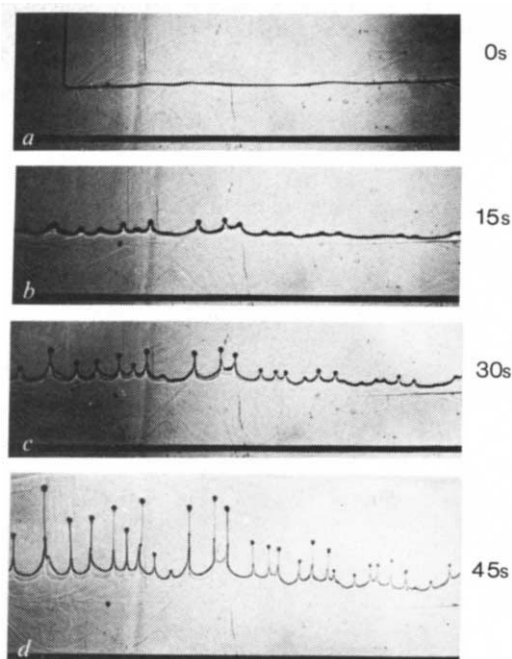


Fig. 1 Gravitational instability of a horizontal line of water/glycerine mixture in a bath of pure glycerine. Kinematic viscosity of the glycerine is estimated as $5.0 \text{ cm}^2 \text{ s}^{-1}$, that of the water/glycerine mixture as $0.015 \text{ cm}^2 \text{ s}^{-1}$, and reduced gravity $g^* = g\Delta\rho/\rho = 260 \text{ cm s}^{-2}$. The dark stripe at the bottom is 0.64 cm diameter tubing, and the initial diameter of the conduit is estimated with a precision optical micrometer to range from 0.16 to 0.24 cm. The spacing of the 25 diapirs in *d* is 39.5 cm for an average of 1.6-cm spacing, giving a ratio of spacing to conduit diameter of 7–10. Marsh argues that the formula for spacing is $d/D = 2\pi/2.15 (\mu_1/\mu_2)^{1/3}$, where μ_1 is viscosity of the upper fluid and μ_2 is viscosity of the conduit fluid with perhaps an order one correction in the coefficient⁴. Our parameter group $2\pi/2.15 (\mu_1/\mu_2)^{1/3}$ is ~ 20 , roughly twice as big, but well within the acceptable range given.

region of partially molten mantle in the Earth will behave in a similar manner and will lead to fairly regularly spaced protrusions from which the melt will ascend to form magma chambers at spreading ridges.

The instability is similar to the classical Rayleigh–Taylor instability in fluid mechanics. It occurs when there is a density inversion, that is a layer of heavy fluid over light. Whitehead and Luther³ reported some experiments and theory on this instability which were relevant when both viscosities were large. They found that the ratio of the wavelength of fastest-growing disturbances to the thickness of the unstable layer was large as the viscosity contrast became large. Thus, a thin layer of relatively low-viscosity fluid can be expected to develop very widely spaced protrusions. Marsh^{4,5} isolated a similar problem, the instability of a ribbon-like, or cylindrical (with its axis horizontal), body of low-viscosity material. The ribbon-like region was produced in the laboratory by allowing black oil or glycerine to rise from a slit in the bottom of a container of glycerine. Marsh suggests that this diapir-genesis occurs under island arcs and is the mechanism that leads to the formation of the magma chambers for the island-arc volcanoes. Noting the regular spacing of magmatic centres across Iceland, Sigurdsson and Sparks⁶ suggest that a similar instability exists at the base of the lithosphere in Iceland.

The suggestion that there is convection at right angles to the direction of relative plate movement is not new (see ref. 7). However, we suggest that an instability due to density change through melting in the mantle beneath the ridge produces upwellings with length scales of the spacing between the mid-points of spreading centre segments. This also implies that axial magma chambers are localized at the midpoints of individual ridge segments.

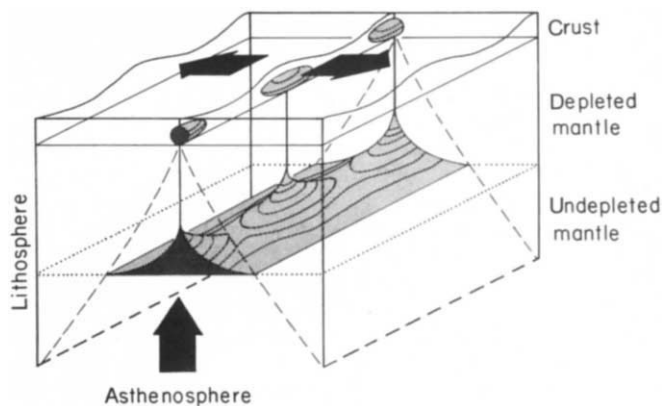


Fig. 2 Viscous asthenosphere rises two-dimensionally beneath the boundary between two spreading lithospheric plates. The lithosphere thickens away from the spreading boundary (dashed lines). Above a certain level (dotted lines), the rising asthenosphere passes through a zone in which partial melt can form which collects at some level below the base of the lithosphere. Due to its lower viscosity and density, the partial-melt zone develops a gravitational instability leading to regularly spaced concentrations of melt which rise to the surface to form crustal magma chambers. The asthenosphere, effectively depleted by this process, must continue to rise viscously and on cooling become lithosphere. The wavelength of the gravitational instability (and the subsequent spacing of spreading centres or transform zones) should also depend on the width of the partial melt zone which is expected to be proportional to the spreading rate.

Evidence for a partially molten zone beneath the base of the lithosphere is seen from regional magnetotelluric measurements in Iceland. These measurements indicate an anomalous conducting layer at a depth of 8–15 km beneath the Neovolcanic Zone which is interpreted as a partially molten zone near the top of the mantle⁸. This zone may correspond to the partially molten region we require in the mantle beneath mid-ocean ridges.

Direct evidence for melt accumulation from a partially molten zone beneath the mid-ocean ridges can be seen in plagioclase-bearing peridotites from oceanic fracture zones. These rocks constitute $\sim 30\%$ of dredged abyssal peridotites⁹ and generally contain $<0.5\%$ interstitial plagioclase which is believed to have largely crystallized from trapped melt^{10–12}. Given the proportion of plagioclase in primitive abyssal tholeiite, it represents $<1\%$ trapped melt in the peridotites, although in rare cases, as locally along the Romanche Fracture Zone, peridotites from one or more dredge haul contain an average of $\geq 6\%$ plagioclase⁹ corresponding to $\sim 13\%$ trapped melt. This is direct evidence for local accumulation of melt in the mantle, which we believe is the result of a Rayleigh–Taylor instability in the partially molten zone and a rapid migration of melt towards the resulting protrusions into the overlying mantle. Green and Hibberson¹³ studied the stability of plagioclase in peridotites at high pressures and found that it was unstable at pressures >8.5 kbar. Based on these phase relations, the zone of melt aggregation represented by the plagioclase-peridotites must lie at <25 km in the mantle.

Abyssal peridotites dredged largely from oceanic fracture zones are generally accepted as the residues of mantle melting which generated abyssal basalts. Almost 70% of these peridotites are plagioclase-free spinel peridotites⁹. Table 1 compares their average composition with independent estimates of undepleted upper mantle composition and a typical primitive abyssal basalt. This shows that melting of the upper mantle would rapidly deplete the solid residue in those elements which occur in far greater abundance in basalt than in the postulated source rock. From the relative concentrations of CaO and Al_2O_3 in Table 1, somewhere between 15 and 20% melt would have to be removed from the postulated parents to produce the observed concentrations of these oxides in the fracture zone peridotites.

Taking all abyssal peridotites together gives an average of 0.9% plagioclase which would correspond to ~1–2% trapped melt. Thus most of the melt which was formed in peridotites dredged from fracture zones must have been drained off. Most of these rocks, however, were dredged from regions in fracture zones where only peridotites were exposed for areas of hundreds, if not thousands, of square kilometres and where little, if any, basalt exists. Accordingly, the melts were not emplaced in the same region as their parental peridotites. We propose that melt formed at depth near fracture zones migrates laterally through the mantle towards an upwelling beneath the centre of the ridge segment rather than that the depleted peridotites were emplaced laterally into the fracture zone after melting beneath the ridge.

Figure 2 summarizes our suggested structure and flow field. The asthenosphere rises viscously beneath the spreading centres. Above a certain level, the rising asthenosphere enters a zone in which partial melt can form. To a first approximation the viscous flow of the asthenosphere is two-dimensional with its axis parallel to the ridge. Thus the rate of partial melt formation is nearly uniform below the ridge axis. The partially molten zone will have relatively low viscosity and density compared with the overlying mantle. The partially molten zone thus develops regularly spaced protrusions centred beneath individual ridge segments. The melt, which can migrate by porous flow, may move more rapidly than the partially molten layer as a whole and concentrate at the tops of the rising protrusions. This would lead to gravitational separation of melt from the mantle and its ascent to form or feed crustal magma chambers. Above the melt aggregation zone, the depleted asthenosphere must continue to rise viscously and, on cooling, become lithosphere.

Young melt concentrations will replenish the older crustal magma chambers. New crust is formed by crystallization of gabbro along the margins of the magma chambers, by the injection of dykes from the magma chambers into the existing crust, both laterally and vertically along the spreading centre axes, and by extrusion of pillow basalts and massive flows from magma chambers and dykes to the top of the crust. Furthest away from the crustal magma chambers, that is, in the transform zones between spreading centres, fewer dykes will be injected and accordingly fewer pillow basalts and massive flows will be extruded, thus producing thinner crust.

This model provides an explanation for the regular segmentation of ocean ridges. Schouten and Klitgord¹⁴ postulated that the sea floor in the slow-spreading North Atlantic (and in other oceans) was formed in a relatively stationary series of spreading centre cells separated by transform-fault zones. From Mesozoic marine magnetic anomalies (155–108 Myr ago) in the western North Atlantic, they estimate a typical separation between spreading centre cells of the order of 50 km, similar to the present distribution of axial valleys on the Mid-Atlantic Ridge. The magnitude of offset between adjacent spreading centres is typically <30 km, can change with time, and can even go through zero and change sign¹⁵. This is inconsistent with an offset-generating mechanism inferred from the rheology of the plate material alone, as the ridges should lose their 'memory' of the fracture zone at 'zero offset'. The stability of spreading centre cells, then appears independent of offsets between them^{14–16}. The model proposed here predicts regular segmentation of ocean ridges, particularly where no large-offset transforms exist. The model also predicts that the crust at small and zero-offset fracture zones should thin, which is consistent with what little is known from seismic studies of these features^{15,17–19}.

Lateral flow of melt towards regularly-spaced protrusions centred beneath individual ridge segments would inhibit flow and mixing of melt in the mantle between ridge segments. Accordingly, melts erupted at each ridge segment will be derived largely from the mantle beneath that segment. This provides a simple explanation for the geochemical discontinuities observed in ridge basalts across fracture zones (see ref. 20) and strongly supports a laterally heterogeneous oceanic mantle. This effect is heightened at large-offset fracture zones where old-cold lithosphere, juxtaposed against the rising asthenosphere, depresses

the partially molten zone¹ and produces a substantial physical barrier to the lateral flow of magmas. We note that our model does not rule out segregation and intrusion of melt to the crust from greater depths in the mantle, nor does it rule out the occurrence of secondary instabilities in the partially molten layer either near fracture zones or laterally away from the ridge axis. We point out, however, that it does predict the presence of geochemical discontinuities in ridge basalts even at zero-offset fracture zones.

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Anaerobic consumption of organic matter in modern marine sediments

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Previous estimates of the global organic carbon (C_{org}) budget have generally considered the anaerobic consumption of C_{org} to be of minor importance. Data for the rates of bacterial sulphate reduction in various morphological zones of the oceans indicate, however, that approximately 14% of the total C_{org} reaching the sediment-water interface is consumed by anaerobic processes. Of this, some 70% is taken up in sediments on the continental shelves and slopes in water depths of less than 1,000 m. Although this represents only a small fraction of the global primary carbon productivity, it may have important consequences for models of the carbon and sulphur cycles.

There is some controversy over the rate of primary productivity in the world ocean. Values ranging from 20 Gton $C yr^{-1}$ (refs 1, 2) to 126 Gton $C yr^{-1}$ (ref. 3) have been reported. In view of recent work which questions the validity of the ^{14}C -technique⁴ and considers contributions from picoplank-

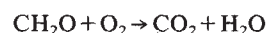
Table 1 C_{org} budget for the top 1 m section of shelf sediments off Mexico (Station 668, depth 140 m, 23°23'N, 106°56'9W)

	C_{org} flux
Primary production in the region	2.5 g m ⁻² d ⁻¹ (= 0.91 kg m ⁻² yr ⁻¹)
C_{org} flux to sea floor (15% of primary production) ¹⁰	0.14 kg m ⁻² yr ⁻¹
C_{org} consumption for bacterial sulphate reduction at the rate of 55.7 µg S per kg sediment per day (ref. 16)	41.8 µg C per kg sediment per day
Thus, C_{org} consumption for anaerobic bacterial sulphate reduction	22.9 g C m ⁻² yr ⁻¹ (= 229 kg C m ⁻² per 10 ⁴ yr)
Residual C_{org} (top 1 m) at 3% C_{org} and 60% water	18 kg C m ⁻²
C_{org} consumption for aerobic respiration for the top 1 m at O ₂ consumption of 10 mol m ⁻² d ⁻¹	1,168 kg C per m ⁻² per 10 ⁴ yr
Hence the total C_{org} consumption in the uppermost metre of sediment (229 + 18 + 1,168) = 1,415 kg C m ⁻² per 10 ⁴ yr.	

The rate of sedimentation is 10 cm kyr⁻¹ (ref. 24), thus 1 m is assumed to have been deposited in 10⁴ yr. The wet density is 1.5 g cm⁻³ and this is therefore equivalent to 1,500 kg m⁻².

ton^{5,6}, chemo- autotrophic⁷ and lithotrophic bacteria⁸, Riley's³ higher value seems the more realistic. Mineralization in the euphotic zone by grazers or chemical oxidation recycles ~60–80% of the particulate organic matter (POM) and, depending on water depth, all or none of its will reach the sea floor.

The POM which ultimately reaches the sediment–water interface will partly be remineralized in the benthic layer⁹ or through the action of burrowing organisms and microbes. It has been estimated that 527,109 moles O₂ are consumed daily in marine sediments¹⁰. This allows POM consumption figures for aerobic processes in bottom sediments to be derived assuming the reaction



to be a reasonable approximation. This yields a value for POM consumption of 2,308 Gton C yr⁻¹.

However, anaerobic processes are also at work in marine sediments and I have attempted to estimate the anaerobic C_{org} remineralization in recent marine sediments.

One way of calculating the anaerobic remineralization of POM is to use the difference in C_{org} or N_{org} values between the top and bottom sections of piston cores and take the resulting decay rate for the two compounds as a measure of anaerobic respiration of POM¹¹. This method, however, has great limitations because even the top sediment layers retrieved in the core material may already contain some biologically inert POM and furthermore, rates of sedimentation may vary considerably between the top and the bottom of the core.

Another approach, which utilizes C_{org} consumption rates, is based on the reduction of manganese, iron and sulphur compounds; estimates of respiration are obtained according to a formula developed by Upsensky and Strakhova^{12–15}. In principle, C_{org} consumption is related to the generation of certain solid phases such as pyrite, but the POM consumed when other oxidized species are reduced, is neglected. Strakhov¹⁵ was the first to draw attention to this oversight. He pointed out that additional factors control POM utilization in marine sediments, such as the release of COOH, CH₄ or NH₃.

Recent biogeochemical findings suggest that not all H₂S generated by reduction of sulphate will enter a solid phase. Some H₂S will migrate upwards or become oxidized. Figure 1 depicts the relationships between various oxidized and reduced sulphur species encountered in representative sediment cores from the Pacific^{15,16}. Sulphate in interstitial water is highly enriched in ³²S, with δ³⁴S values in the range of +15.6 to +18.8‰, compared with a δ³⁴S value of +20‰ for seawater sulphate. This indicates that the sub-surface sulphate maximum (Fig. 1) is caused by the oxidation of isotopically-light metabolic H₂S rather than by a conservative uptake of seawater sulphate during deposition. Analysis of all three forms of sulphur, that is gaseous, dissolved and solid, from anaerobic sediments of Limfjord, Denmark¹⁷ reveals that only 10% of the H₂S generated *in situ* is associated with sulphides and disulphides. The remaining 90% diffuses from shallow muds to near bottom water or on oxidation becomes part of the general sulphur cycle in sediments. From these few but representative examples, it is inferred that reduced sediments, especially those generating free H₂S remineralize more C_{org} during anaerobic microbial early diagenesis than would be expected from the amount of pyrite or other sulphur-containing minerals that are present. These apparent shortcomings can be overcome by using radioactive sulphur- and carbon-isotope techniques to trace and quantify C_{org} remineralization in anoxic sediments^{16–18}.

Bacterial sulphate reduction accounts for 90–95% of the anaerobic remineralized POM^{19,20}. Thus to estimate C_{org} consumption in conditions of anaerobic diagenesis it is sufficient to know the rate at which bacterial sulphate is reduced in sediments. Sulphate reduction can briefly be summarized as follows:



(that is 1 mole S oxidizes 2 moles C) which is a quantitative expression of anaerobic remineralization.

Quantitative data on sulphate reduction in the open sea, in marine sediments, and that obtained in laboratory conditions using radioactive isotope tracer techniques agree very well. Therefore, it is possible to calculate rates of microbial activities for the top metre of modern oceanic sediments and to evaluate C_{org} consumption in conditions of early diagenesis in anoxic media^{11,17,19–21}. In a few instances, knowledge of sedimentation

Table 2 Estimates of global bacterial sulphate reduction in the uppermost metre of oceanic sediments

Geomorphological zone	Depth (m)	Area 10 ⁶ km ²	Intensity of sulphate reduction		Productivity of sulphate reduction (g S m ⁻² yr ⁻¹)	Flux of Σ S _{H₂S} (10 ⁶ tons yr ⁻¹)	C_{org} consumption for sulphate reduction	
			(µg S kg ⁻¹ d ⁻¹)	(mg S kg ⁻¹ yr ⁻¹)			(10 ⁶ tons C yr ⁻¹)	(%)
Shelf	0–50	11(2)	55.6	20.3	26.4	79.2	59.5	16
	50–200	16(3)	37.3	13.6	17.7	53.1	40.0	11
Continental slope	200–1,000	(15)	28.2	10.3	13.4	201.0	151.0	41
	1,000–3,000	(61)	5.5	2.0	2.6	158.6	119.2	32
Foot of slopes, depressions, oceanic bed	>3,000	257	0.97	0.35	—	—	—	—
Total		360 (81)				491.9	369.7	100

Estimates are after refs 16, 18, 20, 21, 25, 26. The figures in parentheses give the areas of biogeochemically active sediments with bacterial sulphate reduction.

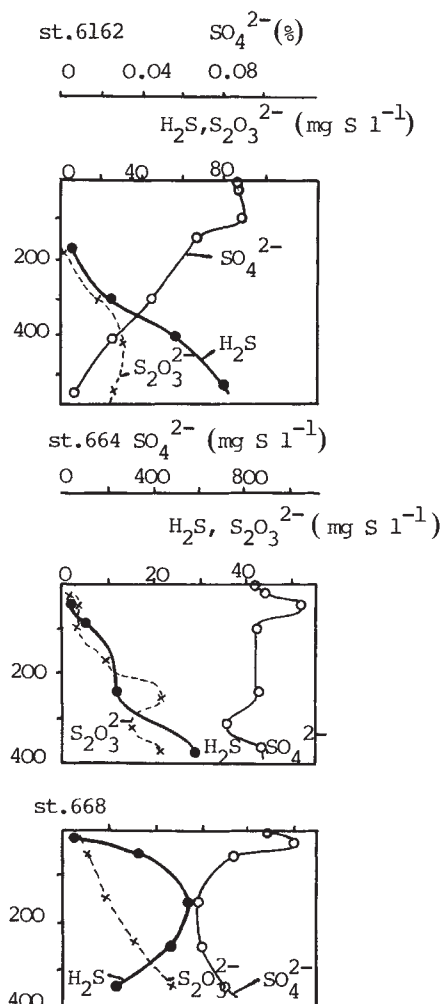


Fig. 1 Depth distribution (in cm) of dissolved sulphur species in pore waters of anoxic oceanic sediments. For location of stations and further details see ref. 24.

rates and the rate of bacterial sulphate reduction (from ^{35}S tracer techniques) allows the calculation of C_{org} budgets. Table 1 summarizes the data for shelf sediments off Mexico. In brief, $\sim 1,400 \text{ kg } C_{\text{org}} \text{ per m}^2 \text{ per } 10^4 \text{ yr}$ enter the marine sediments, of which $<20\%$ are consumed by anaerobic processes. There is a close correspondence between the estimates of C_{org} flux to the sea floor and the total C_{org} consumption during diagenesis.

Experimental data from about 30 stations are compiled in Table 2. This data set covers all critical morphometric zones of the world ocean^{16,17,20,21} and reveals mean global reduction rates for the upper 1 m of the surface sediment layer. About 70% of the total C_{org} consumed anaerobically occurs in sediments down to water depths of 1,000 m. The annual production of metabolic H_2S per square metre sediment of sediment and C_{org} consumed for this process were calculated from the data on the reduction intensity. C_{org} consumption for anaerobic bacterial processes in diagenesis makes up nearly 14% of the total C_{org} arriving at the world ocean bottom (Table 3).

Table 3 Global oceanic C_{org} budget

Process	$10^6 \text{ tons yr}^{-1}$	%
C_{org} consumption:		
aerobic ¹⁰	2,308	83
anaerobic	370	14
C_{org} buried ¹⁶	85	3
Total C_{org} flux to the bottom	2,763	100

Although the above conclusions are reached using comparatively few data points, the global validity of the suggested approach appears to be more meaningful and realistic than pathways taken by previous investigators^{11-15,22,23}. This is because not only solid mineral phases but also a wide variety of gaseous and soluble metabolic products have been considered in the present quantification of anaerobic bacterial processes operating in modern marine sediments.

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A 7,272-year tree-ring chronology for western Europe

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Long tree-ring chronologies provide a unique calendrical record that is of value for archaeological dating, climatic and post-glacial studies. They also form a standard for the calibration of the radiocarbon time scale. The world's longest continuous tree-ring chronology is based on the bristlecone pine (*Pinus aristata* and *Pinus longaeva*) growing in the White Mountains of California¹⁻³. The great age of living and sub-fossil trees of this species enabled a continuous tree-ring sequence of 8,681 years to be established, providing absolutely dated wood samples for the first radiocarbon calibration^{4,5}. We have now established an unbroken west European tree-ring sequence spanning the past 7,272 years.

Figure 1 shows the areas from which the samples are derived. The deciduous oaks (*Quercus petraea* and *Quercus robur*) used for both the German and Irish tree-ring studies show several marked differences from the bristlecone pines. They all grew at low altitude ($<200 \text{ m}$ in Ireland and $<400 \text{ m}$ in Germany, compared with $\sim 3,000 \text{ m}$ for the bristlecone pines), with average life spans of only 150-250 yr compared with 1,000 yr or more for bristlecone pines. Hence, the establishment of this long oak chronology required the linking together of a great number of tree-ring records by the process of cross-dating.

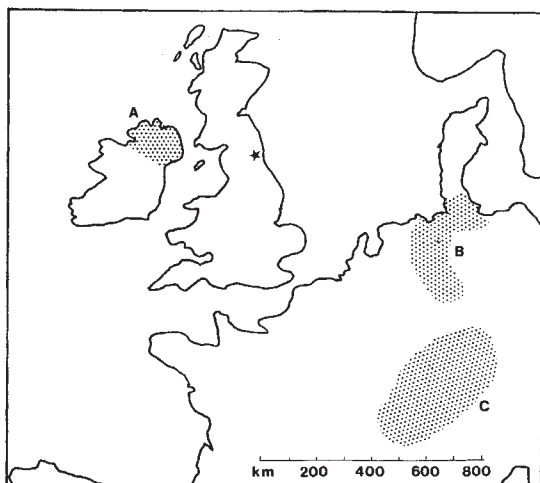


Fig. 1 Map to show the origin of oaks that contributed to the European oak chronology. Area A (north of Ireland), mostly bog oaks; area B (northern Germany), bog oaks and archaeological timbers; area C, southern Germany, mostly river valley oaks. The star indicates the site of Swan Carr in eastern England.

The synchronism of sequences of tree-ring widths is determined by visual and statistical comparison of the patterns of wide and narrow rings. The average growth increment for each year, derived from a number of trees, forms the tree-ring chronology. In both Ireland and Germany, individual ring patterns were cross-dated and merged to form site chronologies. The chronology building process consisted of linking those internally consistent site chronologies, typically of 500–1,000 yr length, to form even longer floating chronologies^{6–8}. Eventually these long floating chronologies were linked to the sequences from living trees to form an absolute time scale. By 1982, a continuous sequence existed in Germany extending back 4,000 yr⁸ (recently extended to 6,000 yr⁹), and in Ireland there was a potential sequence extending back 7,200 yr from the present, with only a single gap between 200 and 13 BC¹⁰.

There is no theoretical basis for judging the geographical distance over which cross-dating will be feasible, so attempts to cross-date the Irish and German sequences were based on previous empirical experience. Successful stepwise cross-dating has been achieved in a west-east transect from eastern France to eastern Austria¹¹, in a north-south direction from Swiss lake dwellings to the south German valleys^{12–14} and up to the southern coastal area of the Baltic Sea^{8,15,16}. It has also been shown that cross-dating is possible from Ireland to Germany by stepwise comparison of chronologies from Ireland to England to Germany^{17,18}. For example, in the modern era, sections of English chronology can be dated to the same calendar year against both the independent Irish and German chronologies. In attempting to establish cross-dating directly between Irish and

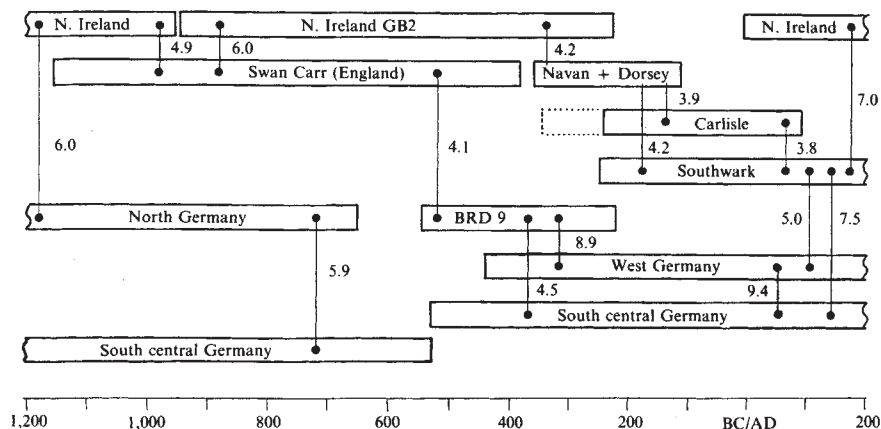
German oak chronologies, one must bear in mind the differences in climatic and ecological conditions. For example, the Irish and north German bog oaks had a different hydrological environment from the river valley oaks of southern Germany and from the dry land oaks used in archaeological sites. However, in spite of these ecological differences and the considerable distances involved, the statistical significance and consistency of the cross-dating is sufficient to ensure that both chronologies are exactly synchronized.

Reliable cross-dating between the north of Ireland and northern Germany for the second millennium BC was established in early 1982 and appeared to place a long floating section of Irish chronology into the exact time span 5218–158 BC. However, by mid-1982, work on timbers from the Roman site at Carlisle in northern England and from Iron Age sites at Navan and Dorsey in Northern Ireland had established a tentative link between the absolute chronologies of the AD era and the young end of the Irish sub-fossil chronology. This specified the end date of the floating Irish sequence as 229 BC instead of 158 BC, as suggested by the link with German chronologies. Baillie¹⁰ pointed out that the dating of the entire German chronology complex earlier than 550 BC was dependent on timbers from a single site, Kirnsulzbach, dated by Hollstein¹⁹. The chronology from this site, which has had its own dendrochronological problems²⁰, seems to have been incorrectly placed. It is now agreed that the German sequence must be broken at 550 BC and the chronology before 550 BC moved back by 71 yr. Once this has been done, consistent cross-dating can be demonstrated between Ireland and Germany at all time periods back to 3000 BC.

A closer look at the critical links within the master chronologies reveals that within the corrected Hollstein chronology there is an overlap of only 33 yr (546–514 BC) and in the south German master chronology an overlap of only 7 yr (546–540 BC). Such overlaps could in themselves never be used as significant links. The weakest link in the Irish sequence is at 250 BC. Here the long floating sequence is linked to the absolute chronology by the Navan–Dorsey series, with overlaps of 130 and 133 yr, respectively. Thus, only by replication between chronologies from the three areas of Ireland, north Germany and south Germany, can final proof of the entire European chronology be provided.

After the German chronology had been broken at 550 BC and the older section moved back by 71 yr, three analyses were carried out to test the replication between the Irish and the corrected German chronologies. The first used the *t*-test²¹ over all the critical overlaps between north Germany and Ireland (Fig. 2). The *t*-test applied here is a rather crude routine test widely accepted as a valuable aid in cross-dating rather than a rigorous statistical test¹⁷. In this program, the correlation coefficient is calculated on the logs of high-pass filtered series and *t* calculated from this. The values of *t* cannot be converted to probabilities because allowance is not made for autocorrelation. The significance of the test in the present study is that in

Fig. 2 Critical links between chronologies from Ireland, England, north and south Germany over the period AD 500 to 1200 BC showing the weak link at 550 BC in the German sequence and at 250 BC in the Irish sequence. The numbers shown beside the links are *t*-values calculated according to Baillie and Pilcher²¹. The unpublished Southwark chronology was provided by Dr I. Tyers. The West German chronology is from Hollstein¹⁹.



all but one of the cases illustrated in Fig. 2 (the exception being Navan-Dorsey and Southwark), the t -value quoted is the highest value for all possible positions of overlap of the two series. The t -test should be seen as complementing the visual cross-dating.

In addition to the t -test, the percentage of parallel variation, GL% (Gleichlaufigkeits value²²), provides a powerful test of synchrony when used on long time series. This simple non-parametric test derives the first differences of the two series and then determines what percentage of the total common years of the two series has the same sign. A test of the south German chronology against the Irish chronology over a total of 4,700 yr gave a GL% of 54.0%, significant at the 99.99% level. If the 1,582 signature years in the south German chronology are tested against the same years in the Irish sequence, the GL% rises to 56.1%, again significant at the 99.99% level. The Irish sequence and the three north German sequences give GL% values of 57.0, 56.8 and 55.6%, all significant at the 99.99% level.

A third confirmatory test was carried out between the Irish and the north and south German chronologies. The long chronologies were split into 400-yr sub-periods, each staggered by 100 yr, and tested using the GL% test. Consistent values (all significant at least at the 95% level, many at 99.99%) were obtained over the entire period from 2000 BC to AD 500. As these correlations are maintained across the weak links at 550 BC and 250 BC, it is possible to claim that a fully replicated European chronology complex exists back to the third millennium BC. The Irish chronology is continuous and internally replicated back to 5289 BC.

Having established on tree-ring grounds that the German chronologies before 550 BC must be moved back by 71 yr, the observed discrepancy between the radiocarbon measurements on bristlecone pine and German oak, found to be between 69 and 72 yr²³, can now be explained. The construction of over 7,000 yr of European tree-ring chronology means that all of the radiocarbon calibration measurements on European oak are now anchored to a calendrical time scale and can be compared directly with measurements on bristlecone pine. The high precision results reported by the Belfast laboratory in 1983 used this time scale^{25,26}. The other implications of the completion of such long sequences of tree rings have yet to be assessed. An increasing number of archaeological sites, particularly in central Europe, can now be given definitive calendrical dates¹²⁻¹⁴. Extension of the European chronology further into the early post-glacial is currently under investigation, and there is a possibility of further replication using another German oak chronology established at the Göttingen laboratory²⁴. The long tree-ring sequences clearly have potential for long-term palaeoclimatic studies not only by palaeoecological²⁷ and climatological^{28,29} interpretation of their growth patterns, but also by their use for radiocarbon³⁰ and stable isotope studies³¹.

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Identification of rat γ atrial natriuretic polypeptide and characterization of the cDNA encoding its precursor

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Diuretic and smooth muscle-relaxing peptides, designated atrial natriuretic peptides (ANPs), have been identified in human¹⁻³ and rat⁴⁻¹⁰ atrial tissues and implicated in the control of fluid volume and vascular function^{11,12}. Recently, cDNAs encoding the human² and rat¹³⁻¹⁵ ANP precursors have been sequenced. We previously isolated from human tissue a natriuretic peptide of molecular weight (MW) 13,000 (γ -hANP) comprising 126 amino acid residues, the largest natriuretic peptide so far identified, and showed that it is directly derived from the 151-residue human ANP precursor by the removal of a signal peptide^{2,3}. We now report the isolation and sequence analysis of a novel rat atrial natriuretic peptide (γ -rANP) of MW 13,000, which derives from the rat ANP precursor. We also report the molecular cloning and nucleotide sequence analysis of the cDNA of the 152-residue rat ANP precursor, which is remarkably similar to the human 151-residue precursor (pre-hANP) except at the C-terminus. Differences in the rat and human precursor nucleotide sequences around the termination codons lead to a difference in processing pattern.

γ -rANP was purified from a side fraction isolated previously^{6,9} using an assay for relaxant activity in chick rectum¹². Early in the purification procedure, the γ -component of MW 13,000 was separated from other lower-molecular weight components on the basis of its weaker basicity by batchwise chromatography on SP-Sephadex C-25 of rat atrial extracts^{6,9}. Gel filtration of the γ -component on Sephadex G-75 yielded crude γ -rANP as a major peak of rectum-relaxing activity eluting in fractions 41-47, corresponding to a molecular weight of ~13,000 (Fig. 1a). Subsequent reverse-phase HPLC yielded purified γ -rANP, a 126-amino acid peptide with similar but less potent natriuretic activity than lower-molecular weight α -rANP⁹ and β -rANP⁶. The yield from 258 rat atria (52.5 g), estimated from amino acid analysis, was 115 nmol which is surprisingly higher than those of smaller rANPs^{6,9} (~1-2 nmol each in the present purification), indicating that γ -rANP is the major natriuretic peptide in rat atria.

Sequence analyses of the disulphide-linked γ -rANP were performed after converting to the reduced and S-carboxymethylated (RCM-) derivative. Direct Edman degradation of the purified RCM- γ -rANP identified the N-terminal sequence (1-41, Fig. 2). The C-terminal sequence of the RCM- γ -rANP, verified by carboxypeptidase digestion^{1,3,6}, was Ser-Phe-Arg-Tyr, which is identical to those of the rANPs so far identified,

Fig. 1 *a*, Gel filtration of fraction SP-II, derived from rat atrial extracts on a calibrated column of Sephadex G-75 (1.8 × 135 cm; flow rate 10 ml h⁻¹; eluent, 1 M AcOH; fraction size, 5 ml per tube). Relative relaxant activity in chick rectum is indicated by black bars. *b*, Reverse-phase HPLC of purified γ -rANP (1.25 nmol). Column: TSK LS-410 ODS SIL (4 × 250 mm; ToyoSoda). Solvent system: linear gradient elution from (A) to (B) for 80 min; flow rate 1 ml min⁻¹; H₂O/CH₃CN/10% trifluoroacetic acid (TFA) = 90:10:1 (A) and 40:60:1 (B) (v/v). *c*, Reverse-phase HPLC of tryptic digests derived from RCM- γ -rANP (2.5 nmol). For trypsinization conditions see Fig. 2 legend. Column: Chemcosorb 3 ODS-H (4.6 × 75 mm; Chemco). Solvent system: linear gradient elution from (A) to (B) for 60 min; flow rate 1 ml min⁻¹; H₂O/CH₃CN/10% TFA = 100:0:1 (A) and 40:60:1 (B) (v/v). Tryptic peptides, designated as T, are numbered sequentially in the order in which they occur in γ -rANP (see Fig. 2). T1' is identical to T1, except that Met residue is oxidized.

Methods: The starting material used was a side fraction, referred to as the γ -component of rectum activity corresponding to MW ~ 13,000, which was obtained in our previous purification of α - and β -rANP^{6,9}. Rat atrial tissue (52.5 g) was extracted from 258 rats and treated as described previously^{6,9} to collect the fraction corresponding to MW < 15,000. After lyophilization, dry material was dissolved in 1 M AcOH, then subjected to batchwise chromatography on SP-Sephadex C-25 (H⁺-form), pre-equilibrated with 1 M AcOH. Successive elutions with 1 M AcOH, 2 M pyridine and 2 M pyridine-AcOH (pH 5.0) yielded three fractions: SP-I, SP-II and SP-III. The γ -component was eluted in fraction SP-II, while the α - and β -components eluted in fraction SP-III. SP-II obtained in this manner was the starting material for purification of γ -rANP. Gel filtration of SP-II was performed on a column of Sephadex G-75. An aliquot from each fraction was bioassayed for relaxant activity in chick rectum (*a*). Fractions 41–47 eliciting the rectum activity were subjected to preparative HPLC on a reverse-phase column of TSK LS-410 ODS SIL (8.0 × 250 mm; ToyoSoda) and rechromatographed. The purity of γ -rANP obtained in this manner was checked by further reverse-phase HPLC (*b*). Bioassay was performed as follows. Chick rectum relaxant activity of the sample was assayed at 37 °C as described elsewhere^{1,12}, using strips of freshly isolated chick rectum. Muscle tone was induced in the rectum strips by adding 2 × 10⁻⁸ M carbamyl choline. Natriuretic and diuretic activities after injection into rats were assayed elsewhere^{1,11}. Three observations were made for each dose.

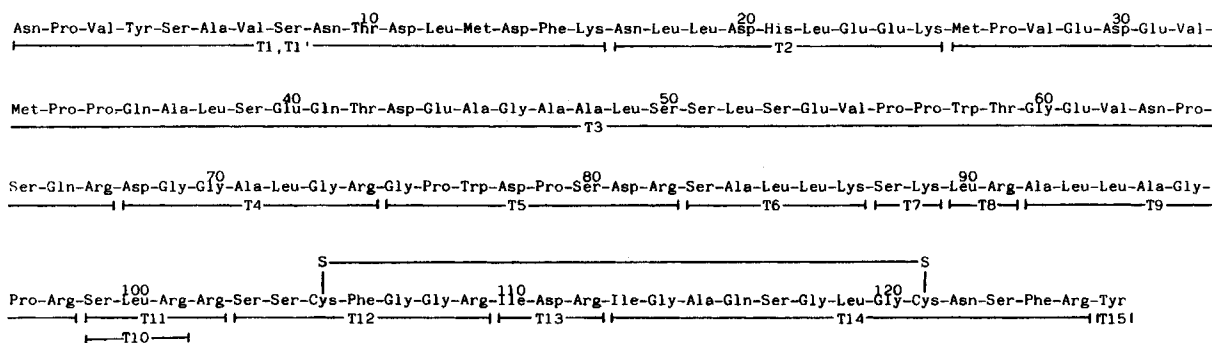
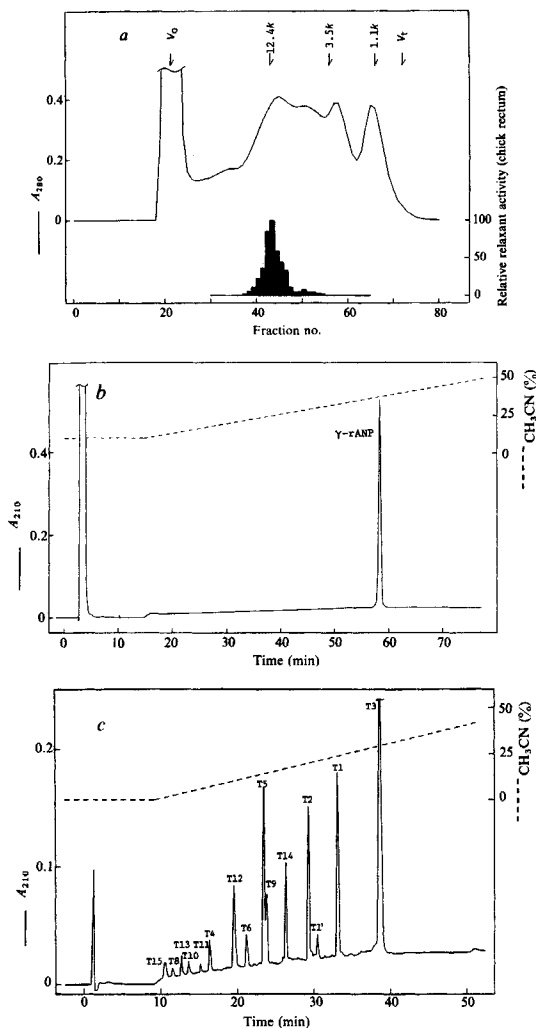


Fig. 2 Complete amino acid sequence of γ -rANP. Sequence analyses were performed with RCM- γ -rANP and a series of tryptic peptide fragments (designated as T). S-carboxymethylation of γ -rANP was achieved by reduction with 50 mM dithiothreitol (DTT) in 0.5 M Tris-HCl buffer (pH 8.5) for 4 h at 37 °C, followed by reaction with 100 mM iodoacetate for 5 min. The resulting RCM- γ -rANP was purified by reverse-phase HPLC. Carboxy-terminal analysis of RCM- γ -rANP (1.0 nmol) was performed by successive digestion with carboxypeptidase A and B (100 ng each; Sigma) in a buffer of 0.2 M N-ethylmorpholine acetate (pH 8.0) at 37 °C. Amino acids released at appropriate time intervals were analysed at the picomole level with a pre-fluorescence labelling analysis system (Waters)^{1,6,9}. RCM- γ -rANP (2 nmol) was digested with 2 μ g of trypsin (tosylphenylethylchloromethyl ketone-treated; Worthington) in 25 μ l of 1% ammonium bicarbonate (pH 8.0) at 37 °C for 3 h. Tryptic peptides were separated by reverse-phase HPLC (Fig. 1c). Sequence analyses of RCM- γ -rANP and its tryptic peptides were performed by stepwise Edman degradation, using a gas-phase automated sequencer (Applied Biosystems 470A), coupled with HPLC identification of the resulting PTH-amino acids.

implying that γ -rANP represents an N-terminally elongated form of the known rANPs.

Further structural information was provided by analysing tryptic peptides derived from RCM- γ -rANP, which were separated by reverse-phase HPLC (Fig. 1c). Overlapping of the sequences of tryptic peptides with the N-terminal (1–41) sequence determined above and the known sequences of 73- (ref. 7) and 48-residue⁶ rANPs made it possible to establish

unambiguously the complete amino acid sequence of γ -rANP (Fig. 2).

We have also cloned and sequenced cDNAs encoding γ -rANP, as described in Fig. 3 legend. Briefly, the hANP cDNA insert which we had previously isolated and analysed was ³²P-labelled by nick-translation and used as a probe for screening a cDNA library constructed from atrial poly(A)⁺ RNA of Wistar Kyoto rats. The nucleotide sequence of plasmid prANP10 which

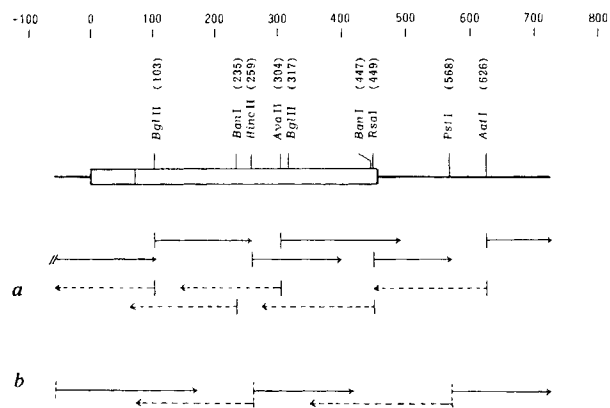


Fig. 3 Sequencing strategy for the cDNA insert of prANP10. The restriction map shows only those restriction sites relevant to the present work; they are identified by numbers indicating the 5'-terminal nucleotides generated by cleavage. □, Sequence corresponding to coding region; ▨, coding region for putative signal peptide. Nucleotides are numbered as in Fig. 4. The direction and extent of sequence determination are shown by arrows (→, upper strand; ←, lower strand). The sites of 5'-end-labelling are indicated by the short vertical lines at the ends of arrows. Slash marks mean that 5'-end-labelling was done on the site located on the vector DNA. Vertical dotted lines mean that the transcription primed by the general primers was started from the sites located on the pUC8 plasmid.

Methods: A cDNA library was constructed by the method of Okayama and Berg²² from 4.2 µg of the vector-primer and 20 µg of poly(A)⁺ RNA isolated from 100 atria of Wistar Kyoto rats as before. Approximately 10,000 transformants of *Escherichia coli* WA802 were obtained from ~1.7 µg of the original poly(A)⁺ RNA; these were screened by hybridization²³ with ³²P-labelled probe at 65 °C for 16 h. The probe was prepared from a 463-bp *Eco*RI-*Pst*I fragment of the cDNA insert of prANP82 which had been cloned from a library of cDNA of human atrial total poly(A)⁺ RNA as described previously³. Positive clones were submitted to cleared lysate analysis^{3,24}, and we confirmed that the three clones (prANP10, 11 and 12) which had a long cDNA insert contained the α-rANP coding sequence by hybridizing with tetradecamer pools (oligo I and II), representing all possible complementary sequences corresponding to α-hANP(12-16), which were used to probe cloned cDNA encoding the human precursor³. The plasmid prANP10 was used for further sequencing as described previously³: a, Maxam-Gilbert method²⁵; b, method of Sanger *et al.*²⁴, after cloning three fragments of the insert (*Pst*I-*Hinc*II-*Pst*I and *Pst*I-*Pvu*II, generated by digestion with *Pst*I and *Pvu*II endonucleases) into plasmid pUC8. (The Okayama-Berg primer-vector carries *Pst*I and *Pvu*II sites.)

had the longest insert (782 base pairs (bp), excluding the poly(A) sequence), was found to contain the entire coding sequence for γ-rANP (Fig. 4). The N-terminal 24 amino acids of the rat ANP precursor (pre-rANP), share characteristic features with the signal sequences generally found in presecretory proteins^{16,17}. It has many hydrophobic amino acids, indeed residues S10-S19 are all hydrophobic, forming a hydrophobic core¹⁸. The apparent cleavage site for signal peptidase is after residue 24, alanine, which often occurs before cleavage sites for other signal peptidases¹⁸. The sequence encoding γ-rANP of 126 amino acid residues, immediately follows the putative signal peptide sequence and ends at residue 150, a tyrosine (amino acid 126), which is connected to the extra C-terminal Arg-Arg sequence. Note that in pre-hANP, translational termination occurs just after the last residue, a tyrosine. Interestingly, 15 nucleotides downstream, the codon for the C-terminal tyrosine of pre-hANP (position 126) is identical to that of pre-rANP, except for a single base replacement at the first nucleotide, which converts the termination codon TGA in pre-hANP to the codon for Arg in pre-rANP. Thus, translation of pre-rANP continues two amino acid residues beyond Tyr 126 and ends at the other termination triplet, TAA. The absence from rat atrial extracts

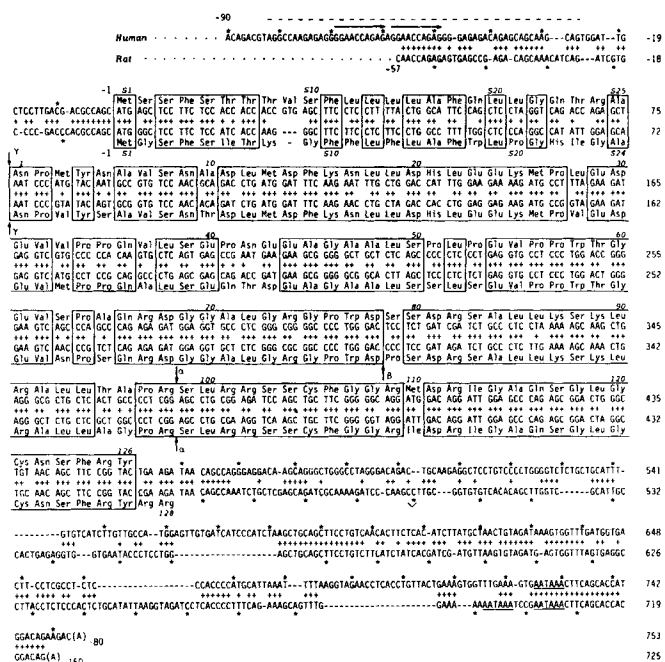


Fig. 4 Nucleotide and deduced amino acid sequences of the cDNA for pre-rANP, compared with those of pre-hANP cDNA³. The nucleotide sequence of prANP10 was determined as described in Fig. 3 legend; the predicted amino acid sequence is shown under the nucleotide sequence. The nucleotide and amino acid sequences of the pre-hANP cDNA³ are shown above those of the pre-rANP cDNA for comparison. Nucleotide residues are numbered (Roman type) in the 5' to 3' direction, beginning with the first residue of the ATG triplet encoding the initiating methionine. Nucleotides 5' of residue 1 are indicated by negative numbers. Amino acids are numbered in italics starting from the initiating methionine and the N-terminal residues of γ-ANPs; S before the number denotes the putative signal sequence. Sequences are aligned to give maximum homology with the introduction of minimal deletions. Deletions are indicated by short horizontal lines on a one-to-one basis. Identical amino acids and the triplets encoding them are boxed; identical nucleotides are marked by +. Asterisks above or below nucleotides of untranslated regions are marker nucleotides. The broken line at the top indicates a sequence which has no thymidylate residues; horizontal arrows indicate direct repeats. Vertical arrows labelled with Greek letters indicate cutting sites used to generate the respective ANP subclasses. AATAAA sequences are underlined. Poly(A) stretches and their approximate chain lengths are shown by (A) and suffixes.

of the peptides that terminate in the Arg-Arg sequence suggests that the C-terminal Arg-Arg is removed from the 152-residue precursor at an earlier stage after synthesis to produce γ-rANP of 126 amino acids. The difference is not due to an error during reverse transcription because the insert of another plasmid (prANP12), which is shorter than that of prANP10, was found to have the same sequence.

The human ANP precursor contains no glycosylation sites¹⁹. In contrast, the rat precursor contains the tripeptide sequence Asn-Pro-Ser, which is regarded as a possible glycosylation site, at positions 63-65 (Fig. 2). However, no glycosylation at Asn 63 was detected in γ-rANP.

There is remarkable homology between the human and rat γ-ANPs in both the nucleotide (85.2%) and amino acid (86.5%) sequences. The amino acid sequences of the putative signal peptides are only 52% homologous, while nucleotide sequence homology is 70.7% if the precursor sequences shown in Fig. 4 are aligned to allow maximum homology. The 5'- and 3'-untranslated regions are very similar. The eight nucleotides upstream of the initiator codons are identical, including the A in position -3 (ref. 20), and the 15 residues downstream from the codons for tyrosine 126 are the same except for the one base as discussed

above. The pre-rANP cDNA sequence has two AATAAA sequences, which precede poly(A) addition sites in many eukaryotic mRNAs²¹, 4 nucleotides apart, whereas the pre-hANP sequence has only one, in the long sequence shared by both precursors. The poly(A) stretch starts immediately after this sequence in the case of pre-rANP, and 5 nucleotides after in the case of pre-hANP. In the 5'-noncoding region of the pre-hANP cDNA insert, GGAACCAGAG sequences are repeated only one nucleotide apart in a long thymidylate-minus sequence. AACCAGAG is also present in pre-rANP cDNA at similar sites, but whether it is repeated or not is unclear because of the apparent incomplete reverse transcription of the mRNA.

Despite the remarkable structural similarities between the

human and rat ANP precursors, their processing is strikingly different. In the human, only three distinct ANPs, α - (28 residues), β - (56 residues) and γ -hANP (126 residues), have been identified¹⁻³. In rat atrial tissue, however, a variety of low- and intermediate-size natriuretic polypeptides (73, 48, 35, 33, 32, 31, 28, 25, 23 and 21 amino acids long) varying in the length of N-terminal extension, has been identified⁴⁻⁹, although in very low yields. The difference in processing patterns may be a result of the amino acid differences between the rat and human γ -ANPs, which may change their conformation and therefore their susceptibility to the proteolytic enzymes, or, more likely, a result of a difference in the enzymatic systems responsible for the processing.

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Electron microscopic autoradiographic localization of opioid receptors in rat neostriatum

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The regional distribution of opioid receptors in the mammalian central nervous system has been extensively studied by light microscopic autoradiography¹⁻³. Little is known, however, about the fine structural localization of these receptor sites apart from the fact that they are present in both synaptosomal and smooth microsomal subcellular fractions of whole brain homogenates⁴⁻⁶. Previously^{7,8}, using the Met-enkephalin analogue FK 33-824 (Sandoz)⁹, we determined that brain opioid binding sites could be visualized by electron microscopic autoradiography. We now report on the ultrastructural distribution of these sites in the caudate-putamen of the rat. The vast majority of specifically labelled opioid binding sites were found to be associated with axo-dendritic (53%), axo-axonic (18%) or axo-somatic (3%) neuronal membrane interfaces. Only 7%, however, were associated with synaptic junctions. These results suggest that opioids act primarily at non-junctional interfaces on the dendrites, axons and soma of neurones in the neostriatum.

Opioid binding sites in rat brain were labelled *in vitro*, using the monoiodinated opioid pentapeptide agonist FK 33-824 (¹²⁵I-FK)⁸ (see Fig. 1 legend). Film autoradiographs from sections incubated with ¹²⁵I-FK alone revealed a patch-like distribution of bound ligand molecules similar to that previously reported for opioid receptors in rat neostriatum^{10,11} (total binding; Fig. 1a). In contrast, sections incubated in the presence of 1 μ M naloxone showed sparse and homogeneous labelling (non-specific binding; Fig. 1b). Both radioactivity measurements and grain counts performed in light microscopic autoradiographs yielded ratios of specific over total binding of the order of 80%.

In electron microscope autoradiographs from sections incubated with ¹²⁵I-FK alone, most of the silver grains (65.3%) were

found by 50% probability circle analysis^{12,13} to be associated with two or more juxtaposed neuronal elements. Radioactive sources were ascribed by 'line source' analysis¹³ to the apposed plasma membranes (70% of the grains within one half distance of the membranes in a sample of 100 shared grains). However, because of limitations inherent to the autoradiographic technique, it was not possible to determine whether radioactive sources were linked to a particular apposed membrane, or to cell surface rather than to internal membrane components. In sections incubated in the presence of 1 μ M naloxone, silver grains were found predominantly over single neuronal structures and their overall distribution was significantly different from that of total binding. The ultrastructural distribution of speci-

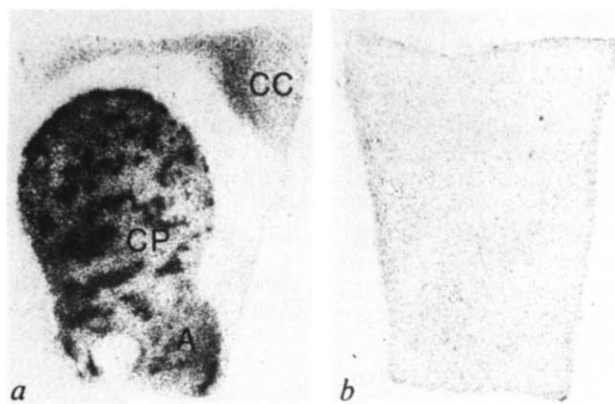
Table 1 Ultrastructural distribution of real and hypothetical grains in autoradiographs from ¹²⁵I-FK-labelled striatal sections

Included within a 50% probability circle	Real (specific) (%)	Hypothetical (%)
Single structures		
Nerve cell bodies	13.1 $\pm$ 1.4	23.3
Axon terminals	1.5 $\pm$ 0.5	12.1
Dendrites	5.9 $\pm$ 3.1	18.7
Apposed structures		
Axon/dendrite:		
Non-junctional	45.8 $\pm$ 3.7	13.0
Junctional	7.1 $\pm$ 0.5	3.1
Axon/axon	17.6 $\pm$ 1.3	9.2
Axon/soma	3.2 $\pm$ 1.1	1.0
Dendrite/dendrite	1.9 $\pm$ 0.5	6.1
Others	3.9 $\pm$ 0.8	13.5
No. of grains counted	1,961	10,039

The distribution of specifically bound ¹²⁵I-FK molecules (real grains) was derived from grain counts performed in sections incubated with ¹²⁵I-FK alone. Values were corrected for nonspecific binding according to the distribution of silver grains in sections incubated in the presence of 1 μ M naloxone. The data are expressed as the mean $\pm$ s.e.m. of five different experiments. Silver grains were ascribed to underlying cellular constituents using a 50% probability circle (diameter = 3.4 $\times$ half distance (HD); HD = 90 nm)¹³. The uniform distribution pattern (hypothetical grains) was generated by means of a transparent overlay displaying a regular array of resolution circles. A χ^2 analysis showed the two distributions to be significantly different with $P \leq 0.005$.

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Fig. 1 Film autoradiographs of 75 μm -thick pre-fixed, vibratome-cut sections from rat neostriatum incubated with 1 nM ^{125}I -FK in the absence (total binding, *a*) or the presence (nonspecific binding, *b*) of 1 μM naloxone, fixed in glutaraldehyde, post-fixed in OsO_4 and dehydrated in ethanols. The distribution of total binding (*a*) conforms with that previously reported for opioid binding sites within the same region^{10,11}. Note the patches of high labelling density in the caudate-putamen (CP) as well as the moderate to strong reactivity of the nucleus accumbens (A) and of the deeper half of layer VI in the cingulate cortex (CC). Nonspecific labelling in the same area (*b*) appears weak and homogeneous by comparison. $\times 7$.



Methods: Adult male Sprague-Dawley rats (150–200 g) were anaesthetized with pentobarbital and perfused for 15–20 min through the ascending aorta with ice-cold 0.1 M phosphate buffer containing 1% tannic acid, 0.75% paraformaldehyde and 0.1% glutaraldehyde. The brains were rapidly removed and serial 70–75 μm -thick sections of the caudoputamen were cut in ice-cold 0.12 M phosphate buffer using a vibrating microtome (Vibratome; Oxford Co.). These sections were incubated with 1 nM monoiodinated ^{125}I -FK 33-824 ($100\text{--}150\text{ Ci mmol}^{-1}$) in 0.05 M Tris-HCl containing 0.25 M sucrose pH 7.4, for 30 min at room temperature. The labelled sections were then rinsed twice in cold buffer, fixed with 4% glutaraldehyde, post-fixed in 2% osmium tetroxide and dehydrated in ethanols. This fixation/dehydration sequence was shown to ensure regionally proportional retention of >50% of the bound radioactivity⁸. After dehydration, the sections were placed in a γ counter for determination of the bound radioactivity and either autoradiographed whole using LKB Ultrofilm or flat-embedded in Epon for light and electron microscopic autoradiography. To assess the proportion and distribution of nonspecific binding, some of the sections were incubated with ^{125}I -FK as above, but in the presence of 1 μM naloxone or non-radioactive FK. Specific binding was defined as the difference between total and nonspecific binding. Projection of film autoradiographs over camera lucida drawings of adjacent Epon-embedded sections allowed precise sampling of receptor-dense areas for high-resolution studies. For light microscopic autoradiography, semi-thin (1 μm -thick) sections were taken from the surface of each block and coated by dipping into Kodak NTB-2 nuclear emulsion. For electron microscopic autoradiography, silver-gold sections were cut from the same blocks, collected on parlodion-subbed slides, double-stained with uranyl acetate and lead citrate, sprayed with carbon and coated with Ilford L-4 emulsion diluted 1:4.

cally bound ^{125}I -FK was in turn significantly different from that of uniformly distributed hypothetical grains (Table 1).

Fifty-three per cent of silver grains corresponding to specifically bound ^{125}I -FK molecules were associated with axo-dendritic interfaces (Table 1). Such interfaces were characterized by a close apposition of axonal and dendritic membranes without intervening glial processes (Fig. 2*a, b*). Axonal elements corresponded in most instances to small varicosities (0.75 μm in mean diameter) containing numerous small, round, electronlucent vesicles (Fig. 2*a, b*) and occasional large granular vesicles. Their dendritic counterparts were mainly shafts of medium to small calibre (Fig. 2*a, b*), but also comprised proximal trunks and spines. In most instances (45.8% of grains counted; Table 1), no synaptic differentiation was present within the resolution circle (Fig. 2*a*). The few labelled contacts exhibiting well defined junctional complexes (7.1% of grains counted; Table 1, Fig. 2*b*) were mostly asymmetrical and were generally established on dendritic spines. Labelled symmetrical junctions were rare

and found solely on dendritic shafts. Several dendritic profiles were labelled at different loci along the plasma membrane (Fig. 2*b*), suggesting that the receptors might be localized on the dendrites themselves rather than on the adjoining axons.

Axo-axonic appositions accounted for the second largest source of specifically labelled ^{125}I -FK binding sites (Table 1). Such appositions included either two axon terminals or one axon terminal and one or more axons. Although a streaming of synaptic vesicles towards the axonal interface was occasionally visible in one of the terminals involved, synaptic differentiations were never observed at the site of contact. One of the terminals, however, was often in synaptic contact with a dendritic profile, generally a dendritic spine (Fig. 2*c*). Previous studies have indicated that selective lesioning of nigrostriatal dopaminergic fibres results in a significant decrease in the number of opioid receptors in rat caudoputamen, suggesting that many of these receptors might be localized on dopamine terminals^{14–18}. The axo-axonic binding sites visualized in the present study may

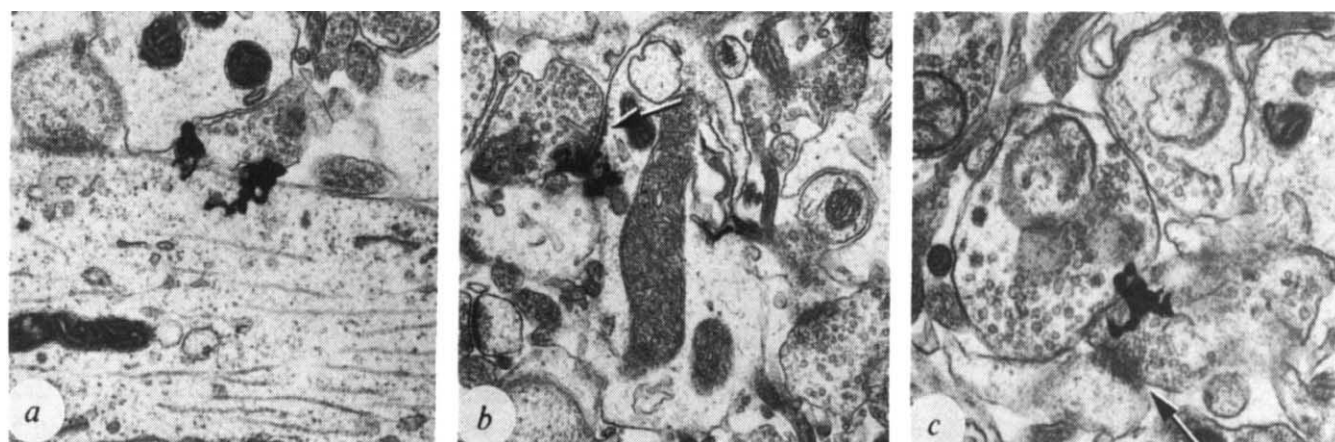


Fig. 2 Electron microscopic autoradiographs from sections incubated with 1 nM ^{125}I -FK alone. The radioactivity is detected in the form of individual silver grains which were ascribed to underlying cellular constituents by means of 50% probability circles (see Table 1). In all cases illustrated here, the 50% probability circle encompasses two neuronal structures and the apposition of their respective plasma membranes. *a*, These two silver grains are associated with a non-junctional axo-dendritic interface involving a small axon terminal filled with numerous small clear synaptic vesicles. $\times 21,000$. *b*, The two silver grains on the left are associated with an asymmetrical axo-dendritic synaptic junction. A third silver grain on the right overlies the plasma membrane of the receiving dendrite. $\times 19,000$. *c*, This silver grain is localized at the interface of two axon terminals. Note the absence of synaptic specialization at the site of apposition. The synapse established on a dendritic spine by one of the terminals involved was not included within the resolution circle. $\times 26,000$. Development, D-19; exposure time, 10 weeks.

correspond to some of these postulated axonal receptors and could thus mediate, at least in part, the demonstrated effects of opioids on the striatal release of dopamine^{19,20}.

Finally, some of the labelled binding sites were associated with axo-somatic appositions (Table 1). Occasionally, a symmetrical synaptic differentiation was present at the site of contact between the axon terminal and the cytoplasmic membrane of the cell. Most of the nerve cells involved were of medium size (12–22 μm in diameter), with a large nucleus showing very little or no indentations and a small rim of surrounding cytoplasm containing very few organelles. These neurones were classified as spiny type 1 neurones, based on cytoarchitectonic analyses of rat neostriatum^{21,22}.

Taken together, the present results strongly suggest that ¹²⁵I-FK-labelled opioid receptors are primarily involved in the mediation or modulation of axo-dendritic, axo-axonic and, to a lesser extent, axo-somatic neural transmission in the rat neostriatum. The identity and site of release of the endogenous ligand(s) acting on these receptors remain to be determined. ¹²⁵I-FK labels both μ and δ opioid receptors^{23–25}, for which enkephalins have been proposed as natural endogenous ligands. Enkephalins could be released by terminals that either possess or are immediately adjacent to ¹²⁵I-FK binding sites, as suggested by the similarity, with respect to size, cytological features and synaptic relationships, between the terminals labelled in the present study and enkephalin-containing terminals previously identified in rat neostriatum by immunocytochemistry^{26,27}. Enkephalins could also be released at a distance from the labelled binding sites and diffuse in tissue to act on a relatively widespread population of receptors; this would account for the apparent lack of correlation between the distribution of enkephalin terminals and opioid receptors in rat striatum (see ref. 28). In any event, endogenous as well as exogenous opioids are likely to act primarily on non-differentiated membrane targets in rat caudate-putamen. Indeed, a major finding of the present study is that only a small proportion of the labelled binding sites was actually associated with synaptic junctions. This is in contrast to α -bungarotoxin^{29–31}, muscarinic³² and benzodiazepine³³ binding sites visualized by electron microscopy or horseradish peroxidase conjugation in rat central nervous system, for all of these have been found to be predominantly, although by no means exclusively, associated with synaptic contacts. That inter-neuronal communication can occur in the absence of membrane specialization has already been postulated for monoaminergic^{34,35} and peptidergic^{36–38} neurotransmission in the central and peripheral nervous systems. Correlative biochemical and immunohistochemical data³⁹ have indicated that opioids, in particular, may act non-synaptically in rat neostriatum, dorsal horn of the spinal cord and spinal trigeminal nerve. The non-junctional receptors visualized in the present study provide the first direct evidence for such a mode of action.

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Hybrid HLA-DC antigens provide molecular evidence for gene *trans*-complementation

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In the mouse there are hybrid determinants for the immune region (*Ir*) of the genome which contribute to the diversity of class II (*Ia*) antigens of the major histocompatibility complex (MHC) and provide a molecular basis for *Ir* gene complementation^{1–4}. In man, two prominent families of Ia molecule, HLA-DR and HLA-DC (or DS)^{5,6}, have been identified which are respectively homologous to the murine I-E (E_α , E_β) and I-A (A_α , A_β) antigens⁷. Whereas DR antigens consist of a constant α -chain and an extremely polymorphic β -chain⁸, both the α and β subunits of DC antigens are structurally variable when different alleles are compared^{9,10}. The marked differences in the structure of the α - and β -chains of HLA-DC molecules result in electrophoretic variants which allow the haplotype of origin of each subunit to be identified by two-dimensional gel electrophoresis. We report here that gene *trans*-complementation occurs in cells heterozygous for the HLA-D region, resulting in the expression of hybrid HLA-DC molecules in addition to the parental forms. Generation of new HLA-D region hybrid molecules contributes to the qualitative diversity of human MHC class II antigens and has important functional implications in immune regulation.

HLA-DC molecules radiolabelled with ³⁵S-methionine were isolated by indirect immunoprecipitation from detergent-solubilized membranes of three cell lines—SC-CA (DR1 homozygous), GM3163 (DR7 homozygous) and LES (DR1/DR7 heterozygous Epstein-Barr virus-transformed)—and a set of monoclonal antibodies previously reported to recognize HLA-DR and/or HLA-DC determinants was used to isolate and characterize the different HLA-DR and HLA-DC molecules present in the cell lines. Monoclonal antibody Genox 3.53 (ref. 11) recognizes the DC1 antigen¹² expressed on DR1-2 or W6-positive cells. As reported previously, the N-terminal amino acid sequence of the DC1 molecule isolated using this antibody is homologous to those of the murine I-A antigens for both the α - and β -chains^{5–13}. Immunoprecipitation of SC-CA

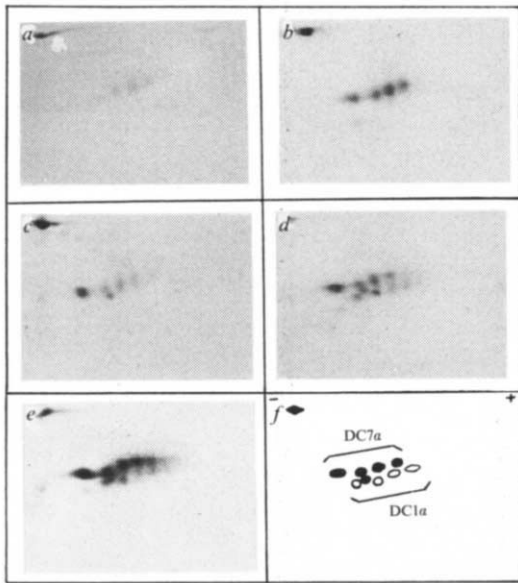


Fig. 1 Comparative IEF two-dimensional PAGE profiles of the DC α -chains from SC-CA (*DR1/DR1*), GM 3163 (*DR7/DR7*) and LES (*DR1/DR7*) cell lines. *a*, The DC1 α -chain isolated from SC-CA using the Genox 3.53 (anti-DC1) monoclonal antibody. 'A' indicates the position of actin (43,000 molecular weight). *b*, The DC1 α -chain from LES immunoprecipitated with Genox 3.53. *c*, The DC7 α -chain from GM 3163 DR⁻ immunoprecipitated with SG 171 or SG 520 is slightly higher and more basic. *d* Illustrates the ability of SG 171 to immunoprecipitate DC7 plus DC1 α -chains in LES DR⁻. The above pattern is identical to that obtained by mixing the DC1 α -chain from SC-CA and the DC7 α -chain from GM 3163 (panel *e*). *f*, Schematic representation of the DC1 α (○) and DC7 α (●) chains IEF20 PAGE patterns.

Methods: Cells were grown in RPMI 1640 supplemented with 10% fetal calf serum and labelled with ³⁵S-methionine in methionine-free medium as described in detail elsewhere¹⁵. The membranes were extracted with 0.5% Nonidet P-40 (NP40). The immunoprecipitated samples were separated by IEF two-dimensional PAGE. The ampholine mixture used in IEF consisted of pH 3.5 ampholine (0.5%) and pH 5–7 ampholine (1.5%). The second dimension was a 10% polyacrylamide slab gel. The panels show portions of autoradiographs of IEF two-dimensional PAGE of the immunoprecipitated proteins. The DC α -chains consist of four or five acidic spots.

(*DR1/DR1*) with Genox 3.53 enabled us to isolate the DC1 molecule; Genox 3.53 does not react with GM 3163. Two other monoclonal antibodies, SG 171 and SG 520, were used to characterize DC7 antigens. SG 171 was originally found to recognize I-A-like molecules from marmoset cell lines¹⁴. However, two-dimensional polyacrylamide gel electrophoresis (PAGE) and partial amino acid sequence data indicate that SG 171 displays an unusual pattern of reactivity for human Ia antigens. In addition to reacting with HLA-DR antigens of all the different alleles, including the *DR7* allele, SG 171 cross-reacts with the HLA-DC molecule present in *DR7* homozygous cells⁶. SG 171 is thus suitable for isolating the DC7 molecule after complete removal of the HLA-DR antigen. This was achieved through several cycles of immunoprecipitation with the monomorphic anti-HLA-DR monoclonal antibody, 1.41 (ref. 15). Antibody SG 520 has recently been reported to react only with DC7 molecules when used on cell lines that are *HLA-DR7* homozygous¹³. In the present work, the HLA-DC7 molecules were isolated by immunoprecipitation with SG 520 or SG 171 after depletion of HLA-DR. (We will refer to cell lysates from GM 3163 (*DR7/DR7*) and LES (*DR1/DR7*) depleted of HLA-DR antigen as GM 3163 DR⁻ and LES DR⁻, respectively.)

The immunoprecipitated HLA-DC antigen was analysed by two-dimensional PAGE as described previously. The use of non-equilibrium pH gradient electrophoresis in the first dimension provides a good separation of the β -chains of both

the HLA-DR^{8–15} and HLA-DC molecules^{6–14}. However, the α -chains were poorly resolved by this procedure^{6–14}, and we therefore used electrophoretic conditions known to maximize resolution of the acidic area of the gel. Analysis of the DC α -chain by isoelectrofocusing (IEF) two-dimensional PAGE in place of non-equilibrium pH gradient two-dimensional-PAGE revealed an acidic series of four or five discrete spots. This pattern is reproducible for a given DC α -chain. Furthermore, the α -chain species could be precisely located according to their position relative to the position of actin, a 43,000-molecular weight protein present in all MHC class II immunoprecipitates^{2–15}. The DC1 α -chains isolated from SC-CA and LES cell lines using Genox 3.53 were indistinguishable by their IEF two-dimensional-PAGE profile (Fig. 1*a,b*). This complete identity was confirmed when a mixture of the two immunoprecipitates was analysed simultaneously on the same gel. Compared with the DC1 α -chain, the α -chain of the DC7 antigen isolated from GM 3163 as described above is slightly higher and more basic (Fig. 1*c*). The DC7 α -chain IEF two-dimensional-PAGE profiles were identical when GM 3163 was immunoprecipitated with antibody SG 520 or when another *DR7* homozygous cell line (Mann) was used. Structural polymorphism among *DC* α alleles has been reported previously using amino acid sequence data, DNA restriction fragment analysis and peptide mapping^{6,10,16}. Based on these results, we searched for electrophoretic variants of the DC α -chains. Because the IEF two-dimensional-PAGE patterns of DC α -chains are reproducible and distinguish between alleles, they can be used as molecular fingerprints to identify the haplotype of origin of the spots and therefore provide a method for typing the *DC* α locus.

Because IEF two-dimensional-PAGE analysis demonstrated haplotype specificity for the DC α -chains we used antibody SG 171 to investigate the DC antigens present in a cell lysate (LES DR⁻) from a *DR1/DR7* heterozygous individual carrying *DC7* on one chromosome and *DC1* on the other chromosome; this antibody would be expected to react only with the DC7 species of DC molecule. However, the PAGE pattern of SG 171-reactive molecules isolated from LES DR⁻ cells revealed a more complex pattern than expected for the DC7 α -chain (Fig. 1*d*); it represented the combined pattern of the DC1 and DC7 α -chains. In fact, the pattern was identical to that obtained when DC1 and DC7 antigen preparation were independently isolated from a *DR1* and a *DR7* homozygous cell respectively and co-electrophoresed on the same gel (Fig. 1*e*). We conclude from this that a monoclonal antibody possessing a biochemical specificity restricted to the DC7 antigen is able to precipitate a DC1 α -chain associated with the DC7 molecule in a *DR1/DR7* heterozygous cell line.

Several hypotheses were investigated to explain the presence of both α -chains. The DC1 α -chain could not carry a determinant recognized by SG 171 because the antibody would then be expected to immunoprecipitate DC1 from SC-CA (*DR1/DR1*), which it does not. This suggests that the DC1 α -chain is precipitated by SG 171 through its association with components of the DC7 antigen. Because non-equilibrium pH two-dimensional PAGE reveals only one β -chain, corresponding to the DC7 β -chain, our observation suggests that the DC7 β -chain freely associates with both the DC7 and DC1 α -chains. Furthermore, depletion of the *DR1/DR7* heterozygous cell lysate with antibody Genox 3.53 removes the complete DC1 molecule (DC1 α , DC1 β) but does not result in a decrease of the DC1 α -chain precipitated with SG 171, thus confirming the presence of an additional molecular species that is independent of the DC1 complex. Because the Ia antigen consists of two closely associated chains, an antibody to either chain would precipitate the α - β dimer. To investigate the possibility that the antibody reacted only with the DC7 β -chain, isolated HLA-DC7 antigens from GM 3163 were precipitated, separated by SDS-PAGE in non-dissociating (unboiled) and dissociating conditions, electrophoretically transferred to nitrocellulose membranes and incubated with dilutions of antibody SG 171 and ¹²⁵I-protein A (Fig. 2). We found that antibody SG 171 bound strongly to the

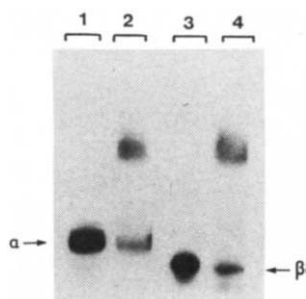


Fig. 2 Detection of DC α -chain specificity of monoclonal antibody SG 171 by electrophoretic transfer of proteins from SDS-PAGE to nitrocellulose. Soluble membrane proteins from unlabelled GM 3163 cells were extracted at 10^8 cells ml^{-1} in 0.5% NP40 Tris-buffered saline (150 mM NaCl, 50 mM Tris, 0.02% NaN_3 , pH 7.0) for 15 min at 4°C. The insoluble material was removed by centrifugation at 100,000g for 60 min. The HLA-DR antigens were retained through eight cycles of immunoprecipitation with antibody 1.41, a monomorphic anti-HLA-DR and *Staphylococcus aureus* A protein¹⁵. The cell proteins depleted of DR reactivity were separated on a 10% polyacrylamide slab gel in non-dissociating conditions (no boiling) and dissociating conditions. Unlabelled polypeptides were transferred to a nitrocellulose membrane. After transfer, the filter was incubated with a 1:1,000 dilution of SG 171 and ¹²⁵I-protein A. DA 147 (ref. 17), an antibody directed against the α -chains (DR and DC), was used as control. DA 147 reacts with the complex (lane 2) and after dissociation only with the α -chain (lane 1). In contrast, SG 171 reacts with the complex (lane 4) and only with the DC β -chain (lane 3). Reactivity with the separated chains (α and β) in lanes 2 and 4 is the result of a low level of chain dissociation which occurs even without boiling the sample.

dimer and to only the β -chain when the complex was dissociated. An antibody directed against the α -chain (DA 147) was used as a control¹⁷.

From the above results, we conclude that in a heterozygous cell line, a DC β -chain can associate freely with the DC α -chain of either maternal or paternal origin, thus generating mixed-haplotype hybrid molecules. Four possible combinations could be generated by the different associations between the α - and β -chains of the DC loci and would therefore be expressed by individual cells. Several laboratories have presented serological evidence that in the mouse, certain Ia determinants exist as hybrid Ia molecules which are expressed on lymphocytes from F_1 animals but are not expressed on cells of either parental strain^{18,19}. Furthermore, combinatorial antigens can function effectively as in the mixed lymphocyte reaction and as restriction elements for T-cell recognition of antigen-specific clones^{1,20,21}. Jones *et al.*² and Silver²² have shown in biochemical studies that one gene product of the I-A region (A_β) and a gene product of the I-E region (E_α) could interact in either the *cis* or *trans* configuration in an F_1 animal. Similar associations occur between the A_α and A_β polypeptides in the appropriate F_1 , as demonstrated by Silver *et al.*³. No convincing serological or biochemical data for an HLA-D region hybrid antigen in humans are available. However, Hansen *et al.*²² observed HLA-D region *trans*-complementation in human T-cell clones that responded to tuberculin only when presented by accessory cells carrying both HLA-D region antigens possessed by the autologous cell donor²². Our finding that DC α - and DC β -chains from a heterozygous cell are capable of associating to form a novel molecule that may carry unique determinants present in the heterozygous cell and absent from both the maternal and parental haplotype may provide a molecular explanation for this. The latter data suggest the presence of new cell-surface molecules functioning as restriction elements independently of whether they originate from the maternal or paternal chromosome. Because the DR α -chains from different alleles are non-polymorphic, the haplotype of origin of the DR α -chain associ-

ated with a given DR β -chain should not be responsible for generating new Ia determinants. In contrast, the variability of the DC α -chain probably creates determinants of mixed haplotype. Such T-cell clones could represent the functional counterpart of the hybrid DC molecules described here.

Our results describe an additional source of structural diversity of the human Ia antigens which are expressed in HLA-D region heterozygous individuals and thus indicate that the number of potential Ia restriction sites is much greater than the number of Ia molecules encoded in the genome.

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Tissue-specific transcription preference as a determinant of cell tropism and leukaemogenic potential of murine retroviruses

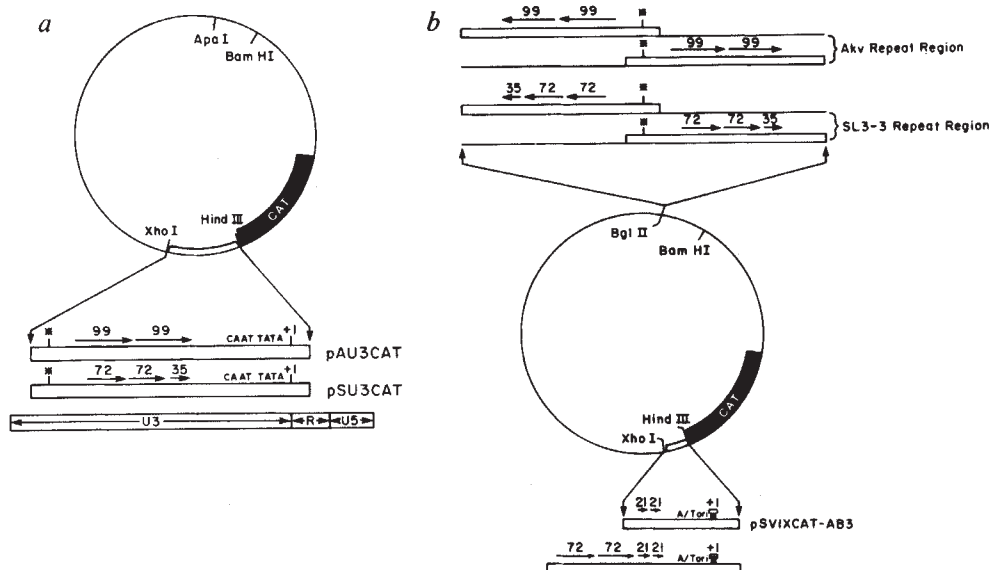
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Inoculation of susceptible strains of mice with the SL3-3 strain of murine leukaemia viruses induces T-cell lymphomas, whereas injection of the Akv strain does not¹⁻³. Recombinant viruses that contain the long terminal repeat (LTR) of the SL3-3 virus and the *gag*, *pol* and *env* genes of the Akv virus are also leukaemogenic⁴. The cell-type specificity of leukaemias induced by viruses containing different LTR sequences is due in part to the ability of the virus to replicate in the appropriate cellular environment⁵⁻⁷. One explanation of the role of the LTR in determination of both cell tropism and leukaemogenic potential is that the LTR encodes tissue-permissive transcriptional elements. We report here that there are differences in the transcriptional activity of the SL3-3 and Akv LTR sequences in different murine cell types, and that the sequences present in the LTR of SL3-3 exhibit significantly enhanced transcriptional activity in T cells compared with the corresponding region of the Akv LTR. The results suggest that transcriptional elements are primary determinants of cell tropism and of leukaemogenicity of these viruses.

To assess the ability of the Akv and SL3-3 LTR sequences to serve as promoter elements in different cellular environments, isogenic constructions⁸ were made in which the U3 regions of Akv (pAU3CAT) and SL3-3 (pSU3CAT) were placed 5' to the chloramphenicol acetyltransferase (CAT) gene⁹ (Fig. 1a). These

Fig. 1 Construction of plasmids used to assess promoter element function (a) and enhancer element activity (b) of murine LTR sequences. **a**, The plasmid subclones harbouring the 5'-LTR of Akv and SL3-3 (pLTR-A4 and pLTR-S3, respectively) were provided by J. Lenz⁴. The *Pst*I-*Ava*I fragment of the LTRs comprises the U3 and a small fraction of the R regions, extending from -443 to +31, relative to the viral RNA start site (designated as +1) for both viruses. This fragment was isolated from each LTR subclone where the natural *Ava*I site was converted to a *Hind*III site using *Hind*III synthetic linkers, and the natural *Pst*I site was converted to a blunt end. The plasmid that served as the recipient vector of the LTR fragments contains the entire CAT gene transcription unit⁹ (CAT coding sequences followed by the SV40 t-intron and polyadenylation signals) under the control of a SV40 early region promoter mutant that carries a deletion of nearly the entire 72-bp tandem repeat element (designated as pSVIXCAT, see below). The SV40 early region promoter, bounded by a synthetic *Xho*I site (5') and a natural *Hind*III site (3'), was removed from pSVIXCAT by successive digestion with *Xho*I, T4 DNA polymerase and *Hind*III enzymes in a fashion whereby the *Xho*I site was converted to a blunt end. The LTR fragments were ligated to the vector fragment containing the CAT gene transcription unit to generate plasmids in which the CAT gene is under the control of the transcriptional elements in the U3 region of Akv (pAU3CAT) or SL3-3 (pSU3CAT). The Akv and SL3-3 U3 regions are identical except for the presence of a single base insertion at nucleotide position 65 (designated by an asterisk) in SL3-3 relative to Akv, and the sequences within the repetitive elements (designated by arrows)⁴. The relative positions of CAAT, TATA and initiation (designated as +1) sequences are indicated. The location and boundary of LTR sequences used in pAU3CAT and pSU3CAT are shown relative to a typical retroviral LTR.



b, To construct plasmids to assess enhancer element activity, a plasmid that carries the CAT gene under the control of the SV40 early region promoter (designated as pSV2CAT⁹) was digested with *Acc*I and *Sph*I to remove the majority of the 72-bp repeat region. The natural *Acc*I and *Sph*I sites were converted to *Xho*I sites using T4 DNA polymerase and a synthetic *Bgl*II linker. Following digestion with *Xho*I and recircularization in the presence of DNA ligase, the resulting plasmid (pSVIXCAT) contains the CAT gene under the control of SV40 promoter sequences extending from -127 to +65 (relative to the 5'-most early start site)²⁹. The structure of this promoter deletion mutant is shown relative to the entire SV40 early region promoter found in pSV2CAT. The repetitive elements (designated by arrows), the origin of replication (ori), the TATA element (denoted as A/T) and early start site(s) (designated as +1) are illustrated. The SV40 promoter sequences present in pSVIXCAT are deficient in enhancer element activity; however, this activity can be restored by linkage of sequences containing enhancer activity to pSVIXCAT at a variety of sites relative to the SV40 early start site(s) (unpublished observations). For this study, a variant of SVIXCAT (designated as pSVIXCAT-AB3) was constructed in which the natural *Apa*I site⁴⁰ located greater than 2.1 kilobases from the SV40 early start site(s) was converted to a *Bgl*II site using T4 DNA polymerase and a synthetic *Bgl*II linker. The LTR subclones, pLTR-A4 and pLTR-S3, were digested with *Sau*3A and *Eco*RI, and a 620-bp *Sau*3A fragment containing 337 bp of U3 region sequences and 283 bp of *Sau*3A fragment D of pBR322 was isolated. The U3 region sequence present in the 620-bp *Sau*3A fragment consists of sequences extending from -443 to -106 (relative to the viral LTR start site in a), and contains the entire tandem repeat region of each virus. The 620-bp *Sau*3A fragment from each LTR subclone was inserted into pSVIXCAT-AB3 in either orientation at the unique *Bgl*II site. All manipulations were done according to standard recombinant DNA techniques⁸.

plasmids were introduced into cultured cells using either the calcium phosphate¹⁰ or the DEAE-dextran methods¹¹. After a transient expression period of 44–50 h, cell extracts were prepared, and the level of CAT enzymatic activity was measured⁹. Previous investigators have demonstrated that for constructions such as those used here, in which the sequences surrounding the RNA start sites are identical, and which differ only in sequences located remotely from the start site⁴, the level of indicator gene activity corresponds directly to the transcription rate^{12–15}. To determine the level of nonspecific and specific expression of the CAT gene in murine cells respectively, parallel transfection experiments were conducted in which cultured cells were exposed to plasmids containing either no eukaryotic promoter element (pSVOCAT)⁹ or the 3'-LTR of Rous sarcoma virus (pRSVCAT)¹⁶ located 5' to the CAT gene.

The level of CAT activity following transfection of these plasmids into murine fibroblast cells is shown in Fig. 2 and Table 1. In NIH 3T3 cells, the level of CAT gene expression of the plasmid containing the Akv LTR sequences is three times that of the plasmid containing the corresponding region of the SL3-3 virus. A similar result was obtained using mouse L cells, another murine fibroblast cell line. From these results, we conclude that in some murine fibroblasts the Akv LTR sequences are more potent than those of SL3-3 in promoting CAT gene expression.

A similar set of experiments was done using murine lymphoid cell lines. In marked contrast to the results observed using

fibroblasts, the level of CAT activity directed by the plasmid containing the SL3-3 LTR sequences was much greater than that directed by the plasmid containing the corresponding Akv sequences in all the T-cell lines tested (Table 1, Fig. 2b). The SL3-3/Akv ratio of transcriptional element activities was 0.3–0.6 in fibroblasts and 6–20 in T lymphocytes. The preferred expression in T-cell lines of the pSU3CAT plasmid relative to that of pAU3CAT is evidently not a consequence of virus infection because the SL3-3/Akv ratio of transcriptional element activity in SL3-3-infected L691 cells (designated as L691.SL3-3) is similar to that found in uninfected L691 cells. Preference for SL3-3 LTR sequences is also observed in primary cell preparations freshly eluted from the thymus of NFS/N mice (Table 1). Evidently, the transcriptional preference of SL3-3 LTR sequences in the established T-cell lines is a general property of T cells. The preferential expression of pSU3CAT is not observed on transfection of the murine B-cell line, M12 (Table 1), suggesting that preferential expression of pSU3CAT is not a general property of all lymphoid cells.

The only differences in LTR sequence between SL3-3 and Akv are located within the tandemly duplicated region located 221–443 base pairs (bp) from the RNA start site⁴, a region shown to include enhancer element functions of other murine retroviruses^{17–28}. This observation raises the possibility that sequences located far from the start site function as primary determinants of the efficiency of promoter element utilization in different cell types. To test this possibility, we measured the ability of the

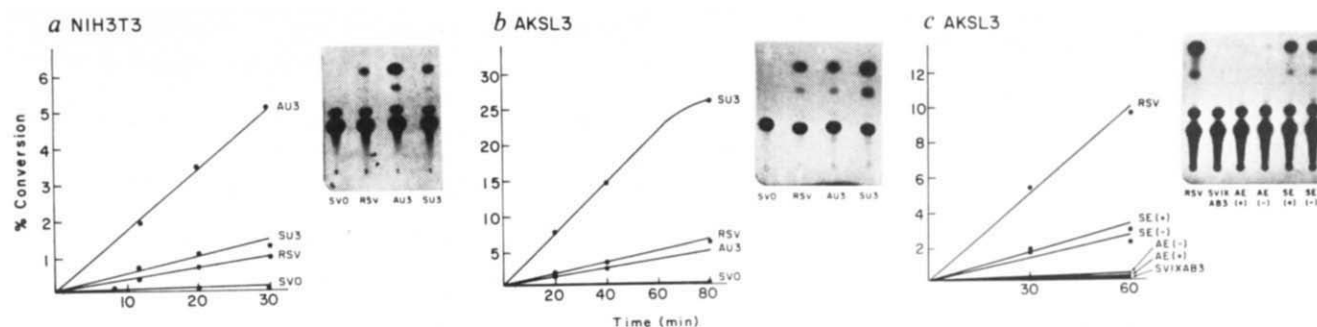


Fig. 2 CAT activity directed by viral LTR sequences in different murine cells. Representative CAT assays for plasmids containing either the Akv U3 region, abbreviated AU3; the SL3-3 U3 region, abbreviated SU3; the 3'-LTR of Rous sarcoma virus, abbreviated RSV; or no eukaryotic promoter element, abbreviated SVO, linked 5' to the CAT gene transcription unit transfected in parallel into cultured cells are shown for NIH 3T3 fibroblasts (a) and AKSL3 T cells (b). c, Representative CAT assay of enhancer element function for plasmids containing either the SV40 promoter sequences lacking enhancer linked sequences, abbreviated SVIX-AB3; the Akv repeat region inserted at the *Bgl*II site 2 kilobases away from the SV40 early start site(s) in the same and opposite transcriptional sense as CAT gene transcription, abbreviated AE(+) and AE(-), respectively; the SL3-3 repeat region inserted at the *Bgl*II site and in the same and opposite transcriptional sense as CAT gene transcription, abbreviated SE(+) and SE(-), respectively; and the 3' LTR of Rous sarcoma virus, abbreviated RSV, linked to the CAT gene and transfected in parallel into cultured cells, is illustrated for AKSL3 T cells. Results are expressed as percentage conversion of ^{14}C -chloramphenicol to ^{14}C -chloramphenicol acetate by extracts containing 400 μg protein in a and b and extracts containing 600 μg protein in c. The insets show typical autoradiograms of CAT assays at 30 min (a), 80 min (b) and 30 min (c). The upper two spots correspond to the two isomers of ^{14}C -chloramphenicol monoacetate while the lower spot is the unreacted substrate, ^{14}C -chloramphenicol.

Methods: Each murine cell line was transfected with an equivalent amount of each plasmid DNA per transfection cocktail. The amount of DNA used for each experiment was within the linear range of optimal CAT activity (data not shown). The NIH 3T3 cell transfections were carried out as described previously¹⁰. The L cell and lymphoid cell transfections were done as described previously¹¹. After a transient expression period (44 h in promoter studies as in a and b; 50 h in enhancer studies as in c), the cells were washed with cold phosphate-buffered saline and resuspended in 200 μl of 0.25 M Tris-HCl pH 7.9. Cell extracts were prepared by three cycles of freeze (-70°C)-thaw (37°C) treatment and centrifugation. *In vitro* CAT enzymatic assays were performed according to published procedures⁹ except that a final concentration of 2.4 mM acetyl coenzyme A was used in each extract assay. Analysis and quantitation of *in vitro* enzymatic assay was done according to previously described procedures⁹.

portion of the U3 region harbouring the tandemly repeated element of SL3-3 and Akv LTR sequences to function as transcriptional enhancer elements in murine T cells. The portion of the simian virus 40 (SV40) early region promoter that governs start site specificity^{29,30} was positioned 5' to the CAT gene (designated as pSVIXCAT-AB3), and the region harbouring the tandem repeat element of either SL3-3 or Akv was linked in *cis*, in both orientations, at a site about 2 kilobases from the SV40 early start sites (Fig. 1b). These plasmids were transfected into murine T cells (the AKSL3 cell line), and the levels of CAT activity were determined relative to pRSVCAT and pSVIXCAT-AB3 (Fig. 2c, Table 2). The level of CAT activity directed by the plasmid containing the SL3-3 repeat region was more than 30 times that of the pSVIXCAT-AB3 plasmid that lacks murine LTR sequences. In contrast to the marked increase in CAT activity observed for the plasmids containing the SL3-3 sequences, the level of CAT activity for those plasmids containing the Akv tandem repeat region sequences was increased only twofold relative to pSVIXCAT-AB3. Similar results were obtained using another murine T-cell line, the SL3B cell line (data not shown).

These experiments demonstrate that in T cells the region harbouring a tandemly repeated element of the SL3-3 LTR behaves as a transcriptional enhancer element^{31,32}; that is, it potentiates increased activity from a remotely located hetero-specific promoter element in an orientation-independent manner. Thus, the enhanced transcriptional activity of the SL3-3 LTR sequences relative to Akv sequences in murine T cells can be attributed to differences in enhancer element function encoded by sequences remotely located in the U3 region.

The increased rate of CAT gene transcription directed by the SL3-3 LTR element in T cells, relative to that of Akv sequences, permits a natural explanation of the T-cell tropism and leukaemogenic potential of the SL3-3 LTR-containing viruses. Replication of retroviruses proceeds via transcription from integrated proviruses. Tissue-specific differences in the rate of transcription of the magnitude observed here, 10–60-fold, may have a profound effect on the extent of viral replication, as such differences might be expected to be magnified as a power function on successive rounds of replication. Consonant with this

model, recent studies indicate that a correlation exists between the cell specificity of viral replication and the type of leukaemia induced by an isogenic set of viruses that differ only in the LTR regions^{5–7}. Thus, it is likely that viral replication, via transcription, in the appropriate cellular context may have a

Table 1 Relative activity of murine LTR sequences as promoter elements in various cell types

Cell type	Promoter element				SL3-3/Akv U3 region activity ratio
	None	RSV 3' LTR	Akv U3 region	SL3-3 U3 region	
Fibroblasts					
NIH 3T3	0.02	1.00	4.94	1.52	0.31
L	0.05	1.00	5.96	3.74	0.62
Lymphoid cells*					
AKSL3	0.01	1.00	0.76	4.50	5.94
SL3B	0.01	1.00	0.51	10.46	20.51
L691	0.04	1.00	0.64	4.09	6.39
L691.SL3-3	0.01	1.00	0.24	1.73	7.20
NFS/N thymocytes	0.85	1.00	0.92	13.57	14.75
M12	0.01	1.00	2.76	1.75	0.64

The CAT activity of each plasmid is normalized relative to the absolute activity of the 3'-LTR of RSV in pRSVCAT within each cell line. Absolute activity for each plasmid in every cell line was calculated from the slope of the line in the initial velocity of CAT enzymatic assay for cell extracts containing 400 μg of protein as depicted in Fig. 2. The absolute activity in any given cell line transfected with each plasmid is expressed in terms of % conversion per h in the *in vitro* assays, and may be obtained quantitatively by multiplying the relative value in the table by the absolute activity value of the same cell line transfected with pRSVCAT. The absolute activity of pRSVCAT in each cell line is given in parentheses: NIH 3T3 (2.06% h^{-1}), L (0.64% h^{-1}), M12 (2.5% h^{-1}), AKSL3 (4.87% h^{-1}), SL3B (2.04% h^{-1}), L691 (0.84% h^{-1}), L691.SL3-3 (2.37% h^{-1}) and NFS/N thymocytes (0.03% h^{-1}). Results shown here reflect an average of at least two independent transfection experiments, and CAT assays performed using extracts containing 400 μg of protein. The range of activities around a particular value varied by not more than 30% of that value.

* Several T-cell lines established from murine T lymphomas were used as recipients and include: AKSL3, a cell line established from a spontaneous tumour of an AKR mouse (the cell line from which the SL3-3 virus was isolated)³⁶; SL3B, a cell line derived from a thymic T-cell tumour of an AKR mouse that had been injected with the SL3 virus³⁷; the uninfected murine T-cell line, L691 (ref. 38); L691.SL3-3, L691 cells infected *in vitro* with SL3-3 virus; and freshly eluted primary thymus cells isolated from NFS/N mice³⁹. The murine B-cell line, M12, was also used as a recipient.

Table 2 Relative activity of murine LTR sequences as enhancer elements in the murine T-cell line AKSL3

Promoter element	Viral repeat region and orientation	Relative activity	Relative-fold enhancement
RSV 3'-LTR		1.000	—
SV40 early region mutant	None	0.01	1.00
SV40 early region mutant	Akv, sense	0.01	1.00
SV40 early region mutant	Akv, antisense	0.02	2.00
SV40 early region mutant	SL3-3, sense	0.37	37.00
SV40 early region mutant	SL3-3, antisense	0.34	34.00

The CAT activity of each plasmid is normalized relative to the absolute activity of the 3'-LTR in pRSVCAT within the T-cell line AKSL3. Absolute activity was determined as described in Table 1. Results shown here are an average of three transfection experiments and CAT assays were performed using extracts containing 600 µg of protein. The range of activities around a particular value varies by not more than 30% of that value. Fold-enhancement for plasmids containing viral repeat regions was determined relative to pSVIXCAT-AB3, the plasmid lacking a viral repeat region.

major role in the induction of a tissue-specific disease by these viruses. Finally, recent studies suggest that murine leukaemia viruses may induce disease by proviral integration adjacent to common loci^{28,33-35}, some of which harbour known oncogenes^{28,34}. Analysis of these integration sites indicates that these loci may be transcriptionally activated by the adjacent viral LTR enhancer element^{28,33-35}. We speculate that an additional, if not simultaneous, characteristic of the LTR of a T-cell leukaemogenic virus is to provide strong enhancer element function sufficient to transcriptionally activate adjacent cellular loci.

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Transferrin receptor on endothelium of brain capillaries

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The blood/brain barrier prevents the passive diffusion of proteins and metabolites from cerebral blood vessels into tissue spaces around neuronal and glial cells. To provide nutrients for these cells, transport mechanisms must exist and indeed have been demonstrated for metabolites¹. We now show that monoclonal antibodies against rat and human transferrin receptors label blood capillaries in the brain but not in other tissues. In the rat this labelling occurs after injection of antibody into the blood, thus the receptors seem to be accessible at the endothelial surface. It is possible that transferrin receptors are expressed on these cells to allow transport of transferrin (and thus iron) into brain tissues.

The MRC OX-26 mouse IgG2a monoclonal antibody is specific for transferrin receptors on the basis that it binds to a detergent-solubilized molecule that can bind ¹²⁵I-transferrin and precipitates a dimer of glycoproteins of apparent molecular weight ~95,000 (ref. 2). Cryostat sections were cut from various rat tissues and stained with MRC OX-26 antibody, followed by peroxidase-conjugated rabbit F(ab')₂ anti-mouse IgG antibody³. One notable reaction occurred with what appeared to be capillaries in the brain; this is shown in Fig. 1a for a 5-µm section from the cerebellum of rat brain and in Fig. 1b for a 30-µm section. The thicker section clearly shows the branched structures, and similar patterns were seen in sections from the brain cortex and spinal cord. Sections from other rat tissues including thymus, lymph node, spleen, heart, kidney, liver, pancreas and small intestine were also stained with OX-26 antibody² but labelling of capillaries was not observed in these sections. In the sections of brain tissue, scattered cells having the morphology of neurones were also stained (for example, see Fig. 1a).

To test whether the antigen recognized by the MRC OX-26 antibody was accessible at the endothelial surface, 2.5 mg of MRC OX-26 IgG was injected intravenously (i.v.) into 4-week-old PVG/c rats and control animals were injected with an equal amount of W6/32 antibody (an IgG2a specific for human histocompatibility locus antigen) or normal mouse IgG. The rats were killed 20 min or 16 h after antibody injection and cryostat sections were cut from their brains and stained with peroxidase-conjugated rabbit anti-mouse IgG antibody. Figure 1c shows clear labelling of the capillaries in a section from an animal given OX-26 antibody 20 min previously, but not in a section from an animal injected with control antibodies. Labelling was still evident 16 h after the injection of antibody, but was weaker than at the earlier time (not shown).

The above results suggest that transferrin receptors are expressed at the luminal surface of the endothelium of rat brain capillaries. Alternatively, the staining could be due to a spurious cross-reaction with an unrelated molecule; this possibility would be excluded if similar reactions were seen with other monoclonal antibodies against transferrin receptors. Several antibodies that react with human transferrin receptors have been described and the antibodies B3/25 (ref. 4), T56/14 (ref. 5) and OKT9 (ref. 6) unequivocally have this specificity. When used to stain sections from two human brains, these antibodies stained the capillaries in both cases. In one case, sections from somatic and

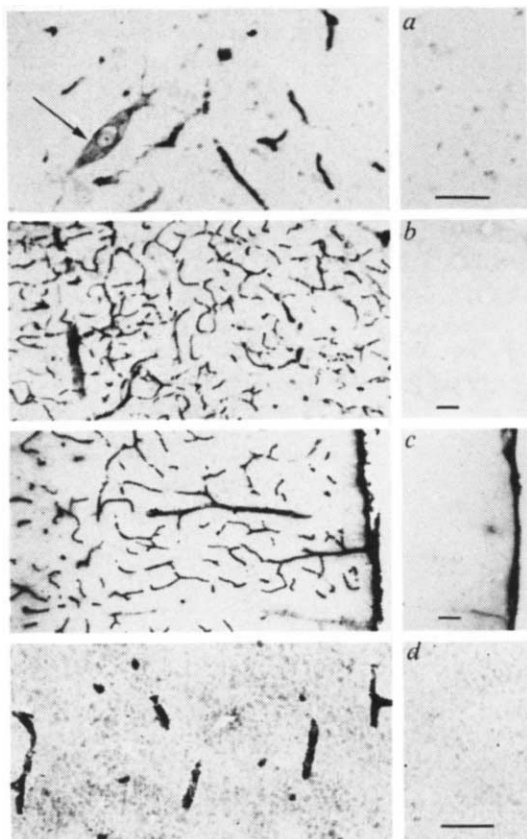


Fig. 1 Staining of sections from rat and human brain with anti-transferrin receptor monoclonal antibodies. *a, b*, Labelling by peroxidase immunohistochemistry of 5- μ m and 30- μ m cryostat sections cut from the cerebellum of 12-week-old PVG/c rats. The sections were cut, fixed and stained with MRC OX-26 monoclonal antibody, followed by peroxidase-conjugated rabbit F(ab')₂ anti-mouse IgG antibody exactly as described in ref. 3. The monoclonal antibody was used as a tissue culture supernatant from OX-26 hybridoma cells. The control shown at the right of each section comprised an adjacent section stained with an antibody that did not react with rat tissues (IgG1 or IgG2a control antibodies were equally unreactive). The arrow in *a* indicates a labelled neurone. *c*, A 25- μ m section from the cerebral cortex of a rat which had been given 2.5 mg of OX-26 IgG i.v. 20 min before killing. This section was stained with peroxidase-conjugated rabbit F(ab')₂ anti-mouse IgG antibody without any first incubation with monoclonal antibody. The control is a section from a rat given 2.5 mg of W6/32 antibody (in ascites fluid) 20 min before killing. *d*, A 9- μ m cryostat section from the cerebellum of a 25-yr-old female human; the tissue was obtained during autopsy (~7 h after death). The section was stained with B3/25 monoclonal antibody and the bound antibody detected by alkaline phosphatase immunohistochemistry, in which the enzyme was cross-linked to the monoclonal antibody by a bridge of rabbit anti-mouse IgG antibody and mouse monoclonal anti-alkaline phosphatase antibody⁹. The sections in *a* and *d* are at a magnification 2.5 times greater than that of *b* and *c*, and the bars in the control sections represent 50 μ m.

visual cortex, basal ganglia, cerebellum, mid-brain and spinal cord were stained and labelling of capillaries was evident in all these areas. Figure 1*d* shows a typical result for antibody B3/25 staining a section of cerebellum. In addition, staining for factor VIII, an established marker for capillaries⁷, was done using the F8.44.20 monoclonal antibody⁸ and the patterns of reaction (not shown) were indistinguishable from those in Fig. 1*d*. In other human tissues, the capillaries were clearly labelled with the anti-factor VIII antibody, but staining of capillaries was not observed with the anti-transferrin receptor antibodies⁵. Labelling of human neurones is not evident in Fig. 1*d*, but scattered neuronal staining was seen in sections of human as well as in rat brain.

The reaction of blood capillaries in human and rat brain with four different monoclonal antibodies suggests strongly that transferrin receptors are expressed on the capillaries, and the results in rat show that these are located at the luminal surface. It is possible that these molecules are involved in the transport of transferrin across the blood/brain barrier.

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Expression of rabies virus glycoprotein from a recombinant vaccinia virus

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Rabies is one of the oldest diseases known to man, but its successful control has remained elusive. Although effective vaccines of tissue culture origin against rabies do exist¹, such preparations are expensive. Live vaccinia virus (VV) recombinants expressing influenza or hepatitis B antigens have recently been used to immunize against these diseases²⁻⁴. We have now used this approach to produce a novel rabies vaccine. We first altered the rabies glycoprotein cDNA⁵ by site-directed mutagenesis and removed the poly(dG) tail. We then aligned the modified cDNA with an early VV promoter sequence inserted within a cloned copy of the vaccinia thymidine kinase gene and transfected this plasmid into VV-infected cells. Recombination between the virus and the plasmid resulted in a recombinant virus harbouring the rabies glycoprotein cDNA. Inoculation of rabbits with the live recombinant virus induced high titres of rabies virus-neutralizing antibodies, and scarification with the recombinant VV protected mice against challenge with street rabies virus.

The rabies virus contains five virus-encoded proteins, only one of which, the glycoprotein, traverses the lipid bilayer envelope. The glycoprotein spike is thus the sole viral component capable of reacting with virus-neutralizing antibodies and eliciting their production. The full-length coding sequence of the ERA strain rabies glycoprotein messenger RNA has been isolated⁵ and its sequence expressed in a bacterial host⁶. Parallel experiments have been described⁷ using the related CVS strain of rabies, but no protection against rabies using the bacterially synthesized material has yet been reported. These data suggest that post-translational modification and/or presentation of the glycoprotein may be important parameters in the use of this antigen in protection against rabies. We have explored the use

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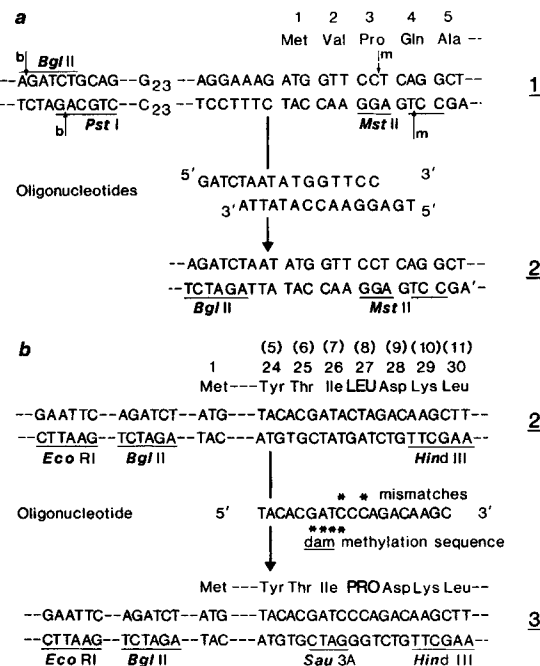
Fig. 1 Restructuring of the rabies glycoprotein cDNA. **a**, The cDNA segment between the *Bgl*III/*Pst*I site at one extremity and a downstream *Mst*II site is replaced by a synthetic double-stranded oligonucleotide adaptor to delete the upstream poly(G)/poly(C) sequence. In **b** the codon for amino acid 8 in the cDNA sequence is changed from CTA to CCA using oligonucleotide-directed mutagenesis. Amino acids of the primary translation are numbered; numbers in parentheses give the amino acid positions in the mature glycoprotein molecule after removal of the N-terminal signal sequence. The precise cleavage sites for *Mst*II and *Bgl*III, respectively, are indicated by 'm' and 'n'. Asterisks indicate mismatches and the *dam* methylation sequence.

Methods: Restructuring the initiation region: In plasmid ptg150(1), the rabies glycoprotein cDNA of Anilionis *et al.*⁵ was introduced, using octameric *Pst*I-*Bgl*III oligonucleotide adaptors¹⁶, into a unique *Bgl*III site inserted at the *Hind*III site of plasmid pBR327 (R.L., unpublished data). This plasmid was digested partially with *Bgl*III and completely with *Mst*II, and the appropriate linear fragment isolated by electrophoresis on a low gelling temperature agarose gel. Oligonucleotides 5'-dGATCTAATATGGTTCC-3' and 5'-dTGAGGAACCATATTA-3' were synthesized using a previously described technique¹⁷. These were hybridized together and ligated between the *Bgl*III and *Mst*II extremities of the purified linear plasmid, permitting recircularization and generating plasmid ptg155(2). Correction of triplet 8: The oligonucleotide 5'-dTACACGATCCAGACAAGC-3' for localized mutagenesis¹⁸ contains two mismatches with the original sequence: a T to C transition correcting the leucine triplet, CTA, to proline, CCA, and an adjacent A to C transversion in the sequence GATA. This generates a *dam* methylation sequence GATC which favours incorporation of the oligonucleotide sequence by selective mismatch correction *in vivo* following primed repair and transfection (see refs 19, 20 for theoretical background to this strategy). The *dam* methylation sequence is itself a restriction recognition site for the enzyme *Sau*3A and permitted rapid identification of correct constructs from digestion profiles of replicative form DNAs. Fragment exchange generated the final reconstructed plasmid ptg155-PRO. Note that the *Eco*RI/*Hind*III fragment was cloned in the reverse orientation of that presented here: the lower strand (complementary to the oligonucleotide) was present in the parental single-stranded M13 bacteriophage recombinant. Hybrid plasmid assembly: Miniplasmid ptg1H was constructed starting from plasmid pML2²¹ (a spontaneous deletion derivative of vector plasmid pBR322 lacking the segment between nucleotides 1,098 and 2,490: probable sequence at deletion boundaries AGCTTCACGGCCAGC, R.L., unpublished results). The *Pst*I recognition sequence was removed by inserting a 692 base pair (bp) *Aha*III-*Aha*III fragment of pUC8²² between two *Aha*III sites of pML2 (removing 19 bp) and the linker-tailing method²³ was used to insert a *Hind*III linker between the *Nru*I and *S*₁-treated *Eco*RI sites of this plasmid (ptg1H). The 4.6 kb *Hin*-J fragment of VV DNA bearing the *tk* gene¹¹ was inserted at the unique *Hind*III site to generate ptg1H-TK. The VV *Sal*-S fragment, carrying the 7.5K promoter²⁴, was isolated in an M13 vector²⁵ as a (shotgun) *Sal*I clone of VV DNA (M13tg*Sal*-S). Here a *Sca*I site lies 33 nucleotides downstream of the transcription start point and 18 nucleotides upstream from the putative initiation codon for the 7.5K gene²⁴. This clone was truncated using the linker-tailing protocol²³ to insert a *Bgl*III linker oligonucleotide 5'-dCAGATCTG-3' between the *Sca*I site and the M13 vector *Bam*HI site after filling the extremity generated by *Bam*HI digestion by DNA polymerase (Klenow fragment) treatment. This eliminates both the *Sca*I site and the downstream *Sal*I (*Acc*I) site, the upstream site becoming unique (M13tg7.5K). The promoter fragment (~270 bp) was excised with *Acc*I and *Eco*RI and ligated between the unique *Cl*aI and *Eco*RI sites (see ref. 26) within the *tk* gene present in ptg1H-TK and generating ptg1H-TK-7.5K, deleting 33 bp (see ref. 27 for an alternative cloning strategy). The *Bgl*III-*Bgl*III fragment from ptg155-PRO was subsequently inserted into the unique *Bam*HI site of ptg1H-TK-P7.5K to generate pVVTGgRAB.

of a VV vector to express the rabies glycoprotein coding sequence.

Expression of exogenous protein coding sequences in VV involves two steps. First, the exogenous coding sequence is aligned with a functional VV promoter and inserted *in vitro* at a site within a (non-essential) segment of VV DNA cloned into a suitable bacterial plasmid replicon. Second, the flanking VV sequences now permit homologous recombination *in vivo* between the plasmid and the viral genome. Double reciprocal recombination results in transfer of the DNA insert from the plasmid to the viral genome, wherein it is propagated and expressed^{2,4,8,9}.

The ERA rabies glycoprotein coding sequence⁵ carries two impediments to its successful translation and expression as an antigen. First, the ATG corresponding to the initiation codon of the mature message lies adjacent to a poly(dG) sequence introduced by the cDNA cloning procedure. This was eliminated by replacing the appropriate cDNA segment by a synthetic double-stranded adaptor, deleting the G/C tail and reconstructing the initiation codon region (plasmid ptg155; Figs 1a, 2). Second, the eighth amino acid of the mature glycoprotein (lacking its signal sequence) of rabies virus ERA and CVS strains is proline^{7,10}, whereas in the cDNA isolated by Anilionis *et al.*⁵, the triplet at this position codes for leucine. The appropriate segment of glycoprotein cDNA was subcloned into a single-stranded bacteriophage vector and oligonucleotide-directed mutagenesis was used to correct the triplet CTA (Leu) to CCA (Pro) (Fig. 1b).



A *Hind*III fragment (*Hin*-J) of the VV genome containing a complete thymidine kinase (*tk*) gene has been used previously to permit recombinational exchange of inserted DNA into the VV genome^{2,3,9}. Transfer of an insert in the *tk* gene to the VV genome creates an easily recognized TK-deficient (TK⁻) virus (Fig. 1). Next, a VV promoter was required to drive the expression of the inserted rabies glycoprotein coding sequence. The promoter of the early VV gene encoding a 7,500 molecular weight (MW, 7.5K) protein has previously been used successfully for a similar purpose^{2,3}, and we inserted this segment into the cloned *tk* gene (Fig. 1 legend). The corrected rabies glycoprotein cDNA was introduced downstream of the (7.5 K) promoter to generate pVVTGgRAB (Fig. 1 legend). This plasmid carries all the elements necessary for transfer of the rabies glycoprotein coding sequence to the VV genome and its subsequent expression.

The strategy devised by Smith *et al.*² relies on *in vivo* exchange between a plasmid bearing an insert within the VV *tk* gene and wild-type viral genome to inactivate the virus-borne *tk* gene. TK⁻ viruses may be selected by plating on a TK⁻ cell line in the presence of 5-bromodeoxyuridine (BrdUrd)⁹. TK phosphorylates BrdUrd to the 5'-monophosphate, which is converted subsequently to the triphosphate. This compound is an analogue of dTTP; its incorporation into DNA prevents correct development of the virus. A TK⁻ virus can, however, replicate its DNA normally and give rise to visible viral plaques on a monolayer of an appropriate TK⁻ cell line.

VV propagates in the cytoplasm of infected cells rather than

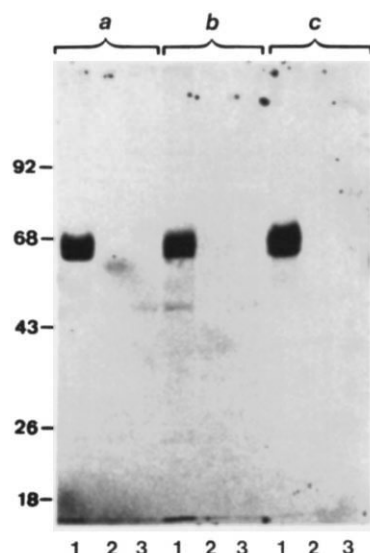


Fig. 2 Immunoprecipitation of proteins synthesized after infection by the VV-rabies recombinant. *a*, Polyclonal antiserum R215 (Wistar) raised against the purified glycoprotein was used; *b*, *c*, identical results were obtained with two monoclonal virus-neutralizing antibodies (509-6 (*b*) and 101-1 (*c*); Wistar) which identify different epitopes on the rabies glycoprotein. The cell monolayers were infected with: 1, recombinant VVTGgRAB-26D3; 2, wild-type VV; 3, no virus. Numbers on the left-hand side represent molecular weight standards ($\times 10^{-3}$).

Methods: Semi-confluent monolayers of LTK⁻ cells ($\sim 10^6$ per plate) were adsorbed with VVTGgRAB-26D3 (50 PFU per cell) for 1 h at room temperature, methionine-free culture medium was added and after 30 min each plate was supplemented with 50 μ Ci of ³⁵S-L-methionine (1,265 Ci mmol⁻¹; Amersham) and incubation continued at 37 °C for 4 h. Cells were collected and resuspended into immunoprecipitation buffer containing protease inhibitors. After sonication and clarification, proteins bound by anti-rabies antisera were recovered by affinity chromatography on protein-A-Sepharose and displayed by gel electrophoresis and fluorography by a modification of a previously described protocol²⁸.

in the nucleus. It is thus unable to take advantage of the host DNA replication and transcription machineries and instead carries in the virion those components necessary for the onset of viral gene expression. For the purpose of generating recombinants, it is necessary simultaneously to infect cells with live VV and introduce the cloned DNA segment of interest by calcium-mediated transfection. However, because the generation of recombinants is limited to those few cells competent for DNA transfection, we used an indirect 'congruence' strategy to reduce the background of non-recombinant parental viruses. This was achieved by using as live infecting virus a ts (temperature-sensitive) mutant of vaccinia unable to propagate at the non-permissive temperature¹¹. When cells infected with a ts mutant in non-permissive conditions are transfected with wild-type virus DNA, viral multiplication occurs only in those cells competent for transfection.

Monolayers of primary chick embryo fibroblast (CEF) cells were infected with VV-Copenhagen ts26 (0.1 plaque-forming units, PFU, per cell), incubated at the permissive temperature (33 °C) for 2 h, then transfected with a calcium phosphate co-precipitate of VV-Copenhagen wild-type DNA ($\sim 0.5 \mu$ g per 10^6 cells) and the recombinant plasmid pVVTGgRAB (3.0 μ g per 10^6 cells). After incubation for 2 h at the non-permissive temperature (39.5 °C), the cells were rinsed and incubated for 48 h at 39.5 °C. Dilutions of ts⁺ virus emerging were reinfected onto a monolayer of mouse LTK⁻ cells at 37 °C and incubated in the presence of BrdUrd (100 μ g ml⁻¹). TK⁻ plaques were obtained from cells that had received the recombinant plasmid, whereas in control cultures without the plasmid no plaques were visible (data not shown).

Table 1 The potential of the recombinant virus to induce rabies-neutralizing antibodies

Post-vaccination day	VVTGgRAB-26D3	Vaccinia wild-type
0	<10	<10
11	10,000	<10
14	>30,000	<10

Rabbits were injected intradermally with 2×10^8 PFU of VVTGgRAB-26D3 virus or with wild-type VV. Inoculations were made in three points on the back. On days 0, 11 and 14, rabbits were bled and sera tested for the presence of rabies virus-neutralizing antibody by a modified rapid fluorescent focus inhibition test¹⁵. Rabies virus was preincubated for 1 h with dilutions of the rabbit antisera and adsorbed to BHK-21 cells. Productively-infected cells were stained after 24 h using a direct immunofluorescence technique. Titres given as the highest dilution at which 50% inhibition of virus infection was observed.

Correct double reciprocal recombination between the hybrid vaccinia-rabies plasmid and the VV genome exchanges the viral *tk* gene for the insert-bearing *tk* gene present on the plasmid. We confirmed the presence of the rabies glycoprotein cDNA by digesting DNA purified from TK⁻ virus with *Hind*III. The 4.6 kilobase (kb) *Hin*-J fragment was absent from these viruses, as we expected, and two new fragments of ~ 1.1 and 5.5 kb revealed the presence of an insert containing an internal *Hind*III site. Southern blotting confirmed that the insert hybridized with rabies glycoprotein cDNA (data not shown) and one recombinant, VVTGgRAB-26D3, was retained. This virus was assessed for its ability to direct the synthesis of rabies glycoprotein in tissue culture cells.

First, monolayers of LTK⁻ cells infected with VVTGgRAB-26D3 were examined for *in situ* immunofluorescence using virus-neutralizing antibodies. Most cells exhibited strong fluorescence associated with the cytoplasmic membrane, whereas non-infected cells and cells infected with the non-recombinant virus showed no fluorescence (data not shown). We infected monolayers of semi-confluent LTK⁻ cells with VVTGgRAB-26D3; polyclonal rabies antiserum as well as two different virus-neutralizing monoclonal antibodies precipitated a protein migrating as a diffuse band of MW 66,000 (Fig. 2). This molecular weight corresponds to the size of ERA virus glycoprotein¹². Furthermore, sera from rabbits immunized with VVTGgRAB-26D3 recognized and bound radiolabelled authentic viral glycoprotein (data not shown). Sera from animals immunized with the non-recombinant VV gave no such reaction. We subsequently examined the potential of the recombinant virus to induce rabies-neutralizing antibodies. Even at the highest dilution of the 14-day antisera, complete neutralization of the virus was achieved.

These results demonstrate that the recombinant VVTGgRAB-26D3 not only induces the production of antibodies reacting with rabies virus, but also that antisera from immunized animals are capable of inactivating rabies virus *in vitro*. This encouraged direct protection testing. Mice immunized by intradermal inoculation of VVTGgRAB-26D3 virus resisted challenge with large

Table 2 Resistance of immunized mice to rabies virus

Immunizing agent	% Protection
None	0
Vaccinia wild-type virus	0
Recombinant virus VVTGgRAB-26D3	100

Female mice (ICR strain) were infected intradermally by scarification with $\sim 5 \times 10^7$ PFU of VVTGgRAB-26D3 or wild-type VV. Four skin scratches were made on the base of the tail using a hypodermic needle and virus suspension rubbed over the abrasion. On day 15 all vaccinated and control animals were infected by intracerebral inoculation with CDC strain tissue-culture adapted street virus (2,400 mouse LD₅₀ units). Test animals were monitored for 2 months post-challenge: unprotected mice succumbed after 8–10 days.

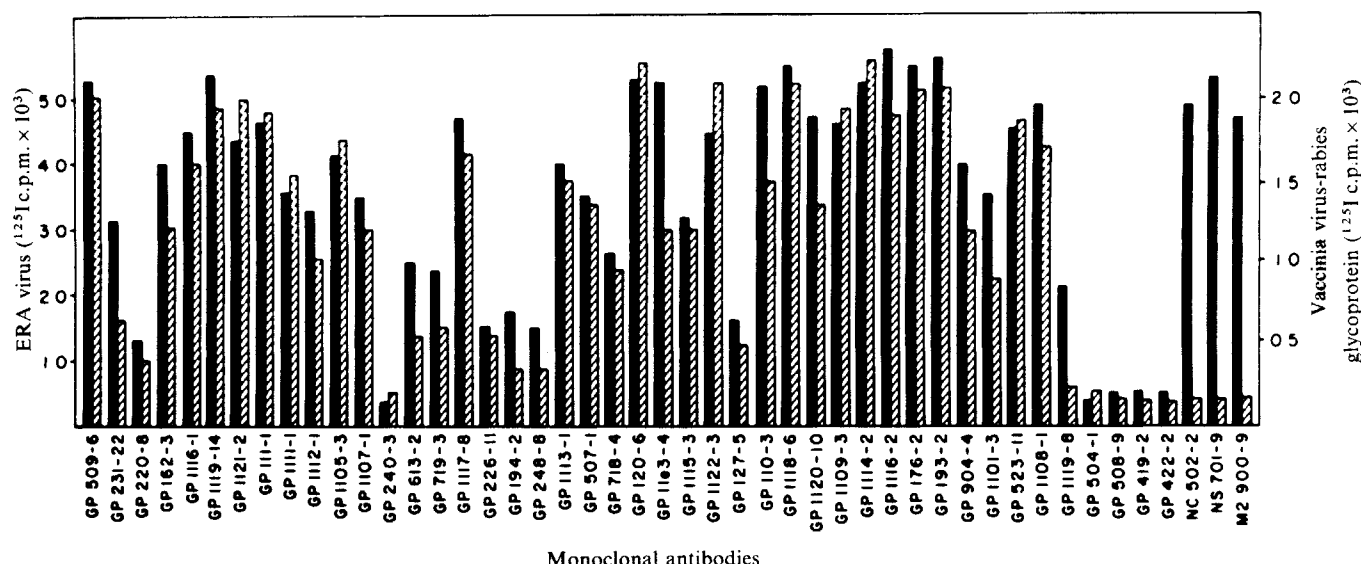


Fig. 3 Comparative binding of VVTGgRAB and ERA virus antigens with a panel of monoclonal antibodies. Solid bars, ERA virus; cross-hatched bars, VVTGgRAB virus.

Methods: Antigens (100 ng) were dried on microtitre plates and treated for 30 min with phosphate-buffered saline (PBS) containing 10% γ -globulin-free horse serum (Gibco). After draining, monoclonal antibody (1:1,000 dilution of ascites fluid; 25 μ l) was added, incubated for 1 h at 37 °C and washed with PBS. Each well then received 25 μ l of 125 I-labelled goat anti-mouse antibodies (30,000 c.p.m., specific activity, 0.5 mCi mg⁻¹). After further incubation (37 °C, 1 h) and washing with PBS, the bottom of each well was cut out and radioactivity determined.

doses of street rabies virus, whereas mice similarly immunized with wild-type VV alone were not protected (Table 2).

To assess the authenticity of the recombinant rabies glycoprotein, reactivity with a panel of monoclonal antibodies directed against rabies glycoprotein and other viral proteins (N, NS and M) was examined. The binding activity of the recombinant glycoprotein with 44 anti-glycoprotein monoclonal antibodies was almost identical to that observed with purified ERA rabies virus, whereas only the ERA virus reacted with anti-N, -NS and -M antibodies (Fig. 3). This demonstrates that the rabies glycoprotein produced by VVTGgRAB virus-infected cells is qualitatively indistinguishable from the native glycoprotein of ERA virus.

Vaccinia virus has been used extensively as a live vaccine to control and eradicate smallpox (see ref. 13 for review); it has been developed as a cloning and expression vehicle for hepatitis B, influenza and herpes antigens and protection has been achieved by vaccination with appropriate influenza- and herpes-VV recombinants^{2-4,14}. We demonstrate here that live VV expressing the rabies glycoprotein is capable of conferring protection against experimental rabies infection. Attenuated viruses such as VV are particularly appropriate vehicles for vaccine production: their preparation and administration can avoid costly procedures involving propagation of the pathogenic agent on cultured mammalian cells and subsequent toxicity testing.

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Molecular cloning and characterization of the HTLV-III virus associated with AIDS

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We recently reported the isolation and characterization of a novel human T-lymphotropic retrovirus, HTLV-III, in patients with acquired immune deficiency syndrome (AIDS) and in those at risk for the disease¹⁻⁴. After extensive sero-epidemiological studies^{3,5,6}, together with numerous virus isolations from these patients^{1,7}, we concluded that HTLV-III is the causative agent of AIDS. Here we report the molecular cloning and characterization of two highly related but distinct forms of the HTLV-III genome. The viral genome is ~10 kilobases long and is detected in HTLV-III-infected cells but not in uninfected cells, including normal human tissue,

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indicating that this virus is exogenous to man. We also demonstrate distant nucleic acid sequence homology between the cloned genome of HTLV-III and those of HTLV-I and HTLV-II. The availability of the cloned HTLV-III genome will now allow an unambiguous comparison of this virus with other retroviruses that also have been associated with the pathogenesis of AIDS⁸⁻¹¹, and moreover, will facilitate the development of diagnostic and therapeutic measures in the treatment of AIDS.

All human retroviruses that have been extensively characterized are lymphotropic, especially OKT4 lymphotropic, and induce formation of multinucleated cells on infection. These viruses also contain a relatively high-molecular weight reverse transcriptase with preference for Mg^{2+} and possess a major core protein of relative molecular mass 23,000–25,000. We named the viruses human T-cell leukaemia viruses, or HTLV, in accordance with recent convention^{12,13}. The first two subgroups of HTLV (I and II) are associated with T-cell malignancies and can transform T cells *in vitro*¹². HTLV-III has many properties in common with HTLV-I and HTLV-II but has cytopathic rather than transforming activity. The crucial step allowing us to isolate and characterize HTLV-III, and to produce sufficient purified viral reagents for serological assays, was the successful transmission of HTLV-III to an immortalized human T-cell line (HT) and to clones derived from this line which were significantly resistant to the cytopathic effects of the virus. This led to the establishment of permanently infected, high-producer cell lines for continuous production of HTLV-III². One of these cell lines, H9/HTLV-III, produces large quantities of HTLV-III and serves as the principal producer cell line for immunological assays used to detect virus-specific antigens and antibodies in sera from AIDS patients. The uninfected parental cell line (HT) and its derivatives (H9 and H4) were negative by all criteria for retrovirus infection, including HTLV-I, HTLV-II and HTLV-III (M.P., in preparation). To clone the HTLV-III genome, we isolated unintegrated viral DNA after acute infection of H9 cells with concentrated HTLV-III and cloned this DNA into a λ phage library to be screened with viral cDNA.

Concentrated virus from the H9/HTLV-III cell line was used to infect fresh uninfected H9 cells at a multiplicity of 50 viral particles per cell and cultures were collected after 4, 10, 15, 24 and 48 h. Extrachromosomal DNA was extracted according to the procedure of Hirt¹⁴ and assayed for its content of unintegrated viral DNA using HTLV-III cDNA as a probe. The synthesis of this cDNA was primed with oligo(dT) and reverse-transcribed from poly(A)-containing RNA of virions that had been banded twice on sucrose density gradients¹⁵. Unintegrated linear viral DNA was first detected after 10 h and was also present at the subsequent time points. Figure 1 shows a Southern blot of the 15-h sampling. A band of ~10 kilobases (kb) in the undigested DNA represents the linear form of unintegrated

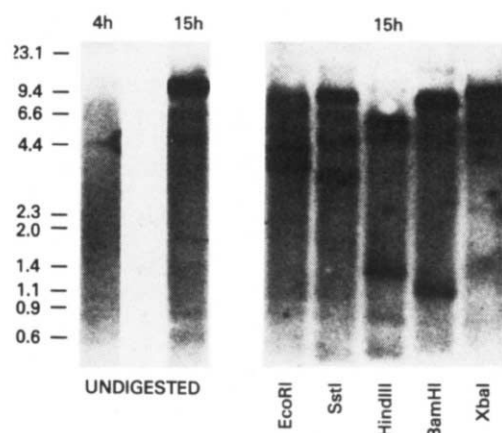


Fig. 1 Southern blot²² analysis of unintegrated HTLV-III DNA. No viral sequences could be detected in the undigested DNA after 4 h. However, a major species of viral DNA ~10 kb long was present at 10, 15, 24 and 48 h, representing the linear unintegrated form of the virus. The figure shows a representative Southern blot of the 15-h sample digested with several restriction enzymes.

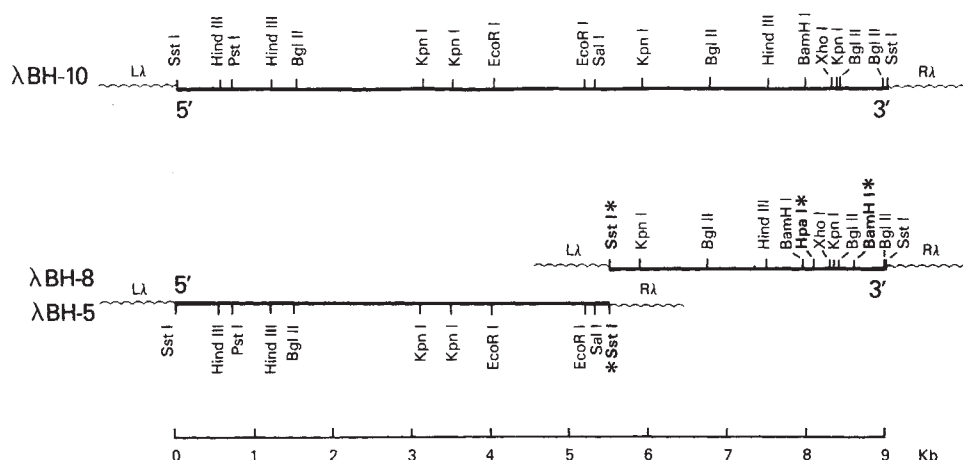
Methods: Fresh uninfected H9 cells (8×10^9) were infected with concentrated supernatant from cell line H9/HTLV-III containing 4×10^{11} particles of HTLV-III. Infected cells were divided into five roller bottles and collected after 4, 10, 15, 24 and 48 h. Low-molecular weight DNA was prepared using the Hirt fractionation procedure¹⁴ and 30 μ g of undigested and digested DNAs were separated on a 0.8% agarose gel, transferred to nitrocellulose paper, and hybridized to an HTLV-III cDNA probe for 24 h at 37 °C in $2.4 \times$ SSC, 40% formamide and 10% dextran sulphate. cDNA was synthesized from poly(A)-selected RNA prepared from doubly banded HTLV-III virus in the presence of oligo(dT) primers²³. Filters were washed in $1 \times$ SSC at 65 °C.

HTLV-III. No closed or nicked circular DNA could be detected at 10, 15 or 24 h, but both forms were evident in small amounts at 48 h (data not shown). The viral genome was not cleaved by *XbaI*, whereas *SstI* generated three predominant bands of 9, 5.5 and 3.5 kb (Fig. 1). We interpreted these bands as representing the genomes of two forms of HTLV-III, both cut by *SstI* in or near the long terminal repeat (LTR), and one having an additional *SstI* site in the middle of its genome. The other enzymes generated a more complex pattern of restriction fragments requiring cloned DNA for further analysis.

Figure 2 shows the restriction map of three clones, designated λ BH10, λ BH5 and λ BH8, which correspond in size to the three *SstI* fragments shown in Fig. 1. Comparison of these maps suggests that λ BH5 plus λ BH8 constitute one HTLV-III

Fig. 2 Restriction endonuclease map of two closely related HTLV-III forms cloned from unintegrated viral DNA. Three recombinant clones (λ BH10, λ BH5 and λ BH8) were analysed and their inserts (9, 5.5 and 3.5 kb, respectively) were mapped with the indicated enzymes. Together they represent two genomic equivalents of HTLV-III that are highly related but differ in three enzyme sites, indicated by bold letters and asterisks.

Methods: Low-molecular weight DNAs pooled from the 15- and 24-h samples were fractionated on a 10–40% sucrose gradient²³. Aliquots of the fractions were electrophoresed on a 0.5% agarose gel, transferred to nitrocellulose paper and hybridized to HTLV cDNA in conditions described in Fig. 1 legend. Fractions containing the unintegrated linear HTLV-III genome shown by hybridization were pooled; the DNA was subsequently digested with *SstI*, then ligated to phosphatase-treated *SstI* arms of λ gtWes- λ B. After *in vitro* packaging, recombinant phages were screened for viral sequences with HTLV-III cDNA^{15,23}.



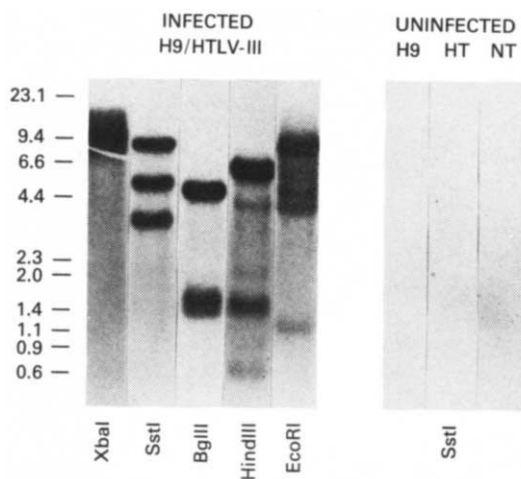


Fig. 3 Demonstration of the presence of HTLV-III viral sequences in the infected cell line, H9/HTLV-III. Both variant forms of HTLV-III defined by differences in *SstI* sites were detected in H9/HTLV-III DNA. No HTLV-III sequences were found in uninfected H9 cells, uninfected HT cells or normal human thymus (NT).

Methods: High-molecular weight DNA (10 µg) was digested with restriction enzymes as indicated and hybridized to the nick-translated phage insert from λ BH10 in the conditions described in Fig. 1 legend.

genome, and λ BH10 another. The two viral forms differ in 3 of 21 mapped enzyme sites, including the internal *SstI* site. As expected, the phage inserts of λ BH5 and λ BH8 hybridize in high-stringency conditions ($T_m = -25^\circ\text{C}$) to λ BH10 but not to each other, as analysed by Southern blot hybridization and electron microscopic heteroduplex analysis (data not shown). To determine the orientation of the three clones, we used as a probe a cDNA clone (C15) containing U3 and R sequences (S.K.A. *et al.*, in preparation); C15 hybridized strongly to the 0.5 kb *BglII* fragment of λ BH10 and λ BH8, orienting this side 3'. Assuming that *SstI* cuts only once in the vicinity of the HTLV-III LTR, the clones λ BH10 and λ BH5/λ BH8 represent two complete genomic equivalents of the linear unintegrated form of HTLV-III that vary in three restriction enzyme sites. However, the viral fragments cloned into λ BH5 and λ BH8 may have been derived from the same or two different viruses.

The presence of two variant forms of HTLV-III in the original cell line was demonstrated by hybridizing the radiolabelled insert of λ BH10 to a Southern blot of H9/HTLV-III genomic DNA digested with several restriction enzymes (Fig. 3); both forms were detected using *SstI*, which generated the expected three bands of 9, 5.5 and 3.5 kb. *XbaI*, which does not cut the provirus, generated a high-molecular weight smear representing polyclonal integration of the provirus, plus a band of ~10 kb. This 10-kb band was also detected in undigested H9/HTLV-III DNA (not shown), indicating that it represents unintegrated viral DNA. The presence of unintegrated viral DNA also explains the 4- and 4.5-kb *EcoRI* fragments seen in both the Hirt and total cellular DNA preparations (Figs 1, 3). Both *BglII* and *HindIII* cut within the LTR and generate the expected internal bands. Several faint bands in addition to the expected internal bands generated by *HindIII* digestion, represent either defective proviruses or other variant forms of HTLV-III present in low copy number.

The absence of HTLV-III sequences from the DNA of the uninfected H9 cell line, the uninfected parental cell line HT and normal human thymus (Fig. 3), demonstrates clearly the exogenous nature of HTLV-III and shows that the virus does not contain human cellular sequences. The same results were obtained using inserts from λ BH5 and λ BH8 as probes.

The availability of the cloned HTLV-III genome also allowed us to evaluate sequence homology between HTLV-III and other members of the HTLV family including HTLV-I and HTLV-II,

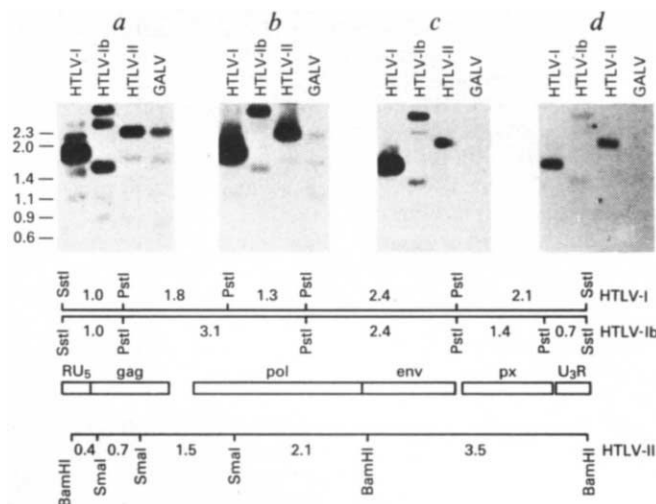


Fig. 4 Sequence homology of HTLV-III to other members of the HTLV family. Schematic restriction maps of HTLV-I, HTLV-Ib and HTLV-II are shown at the bottom, indicating the length (in kb) and location of the generated fragments with respect to the corresponding genomic regions of HTLV-I. LTR, *gag*, *pol*, *env* and *pX* regions are drawn to scale according to the published nucleotide sequence of HTLV-I²⁴. The bands that are most highly conserved as stringency increases correspond to the *gag/pol* junction region of HTLV-I (1.8-kb *PstI* fragment) and HTLV-Ib (3.1-kb *PstI* fragment) and to the 3' part of the *pol* region of HTLV-II (2.1-kb *SmaI/BamHI* fragment) which do not overlap assuming that HTLV-II has a genomic organization similar to that of HTLV-I. Fragments corresponding to *pX* of HTLV-I (2.1-kb *SstI/PstI* fragment) and HTLV-Ib (1.4-kb *PstI* fragment) are only faintly visible at $T_m = -28^\circ\text{C}$ on the original autoradiogram. Digestion of GaLV generates six fragments, none of which hybridizes with HTLV-III in medium or high stringency conditions ($T_m = -42^\circ\text{C}$ and -28°C).

Methods: Subclones of full-length genomes of a prototype HTLV-I (unpublished), HTLV-Ib¹⁶, HTLV-II²⁵ and GaLV (Seato strain)²⁶ were digested with the following enzymes; *PstI* plus *SstI* (HTLV-I and HTLV-Ib); *BamHI* plus *SmaI* (HTLV-II); and *HindIII*, *SmaI* and *XhoI* (GaLV). Four replicate filters were prepared and hybridized for 36 h under low stringency ($8\times\text{SSC}$, 20% formamide, 10% dextran sulphate at 37°C) to nick-translated insert of λ BH10. Filters were then washed in $1\times\text{SSC}$ at different temperatures: a, 22°C ($T_m = -70^\circ\text{C}$); b, 37°C ($T_m = -56^\circ\text{C}$); c, 50°C ($T_m = -42^\circ\text{C}$); and d, 65°C ($T_m = -28^\circ\text{C}$), and subsequently autoradiographed for 24 h.

as well as a variant of HTLV-I (HTLV-Ib) recently isolated and molecularly cloned from a Zairian patient with adult T-cell leukaemia¹⁶. Replicate Southern blots of restriction enzyme-digested clones comprising the complete genomes of HTLV-I, HTLV-Ib and HTLV-II, and of gibbon ape leukaemia virus (GaLV) as a control, were hybridized with the full-length HTLV-III probe (BH10) in relaxed conditions, after which the filters were washed in conditions of low, medium and high stringency (Fig. 4). This experiment demonstrates homology between HTLV-III and HTLV-I, HTLV-Ib and HTLV-II, but not between HTLV-III and GaLV. Hybridization of HTLV-III with other members of the HTLV family could be detected in conditions ($T_m = -42^\circ\text{C}$ and -28°C) where no hybridization to GaLV was seen (Fig. 4c, d). Note that the restriction fragments showing greatest homology to HTLV-III correspond to the *gag/pol* region of HTLV-I and to an apparently non-overlapping portion of the *pol* region of HTLV-II (assuming that the genomic arrangement of HTLV-II is similar to that of HTLV-I). Hybridization to a fragment containing exclusively *pX* sequences in HTLV-Ib (1.4-kb *PstI* fragment) and to the corresponding fragment in HTLV-I containing *pX* and LTR sequences (2.1-kb *PstI/SstI*) was detectable at $T_m = -28^\circ\text{C}$ but was very faint. *pX* sequences of HTLV-II did not hybridize to the HTLV-III probe in the same stringency conditions, nor did fragments containing LTR or envelope sequences of both HTLV-I and HTLV-II.

Overall, these findings using the cloned HTLV-III probe agree with our previous observations using HTLV-III cDNA¹⁵, which also revealed sequence homology, especially in the *gag/pol* regions of the HTLV-I, HTLV-II and HTLV-III genomes. However, we emphasize that HTLV-III is much less closely related to HTLV-I and HTLV-II at the nucleic acid level than HTLV-I and HTLV-II are to each other^{17,18} and that this homology is most evident in the *gag/pol* region of these viruses under stringent hybridization.

Thus, we have molecularly cloned two closely related but distinguishable genomic equivalents of HTLV-III from the H9/HTLV-III cell line, which has been the principal source for all viral reagents used in studies of the sero-epidemiology of HTLV-III in AIDS patients¹⁻⁷. Note that this virus from the H9/HTLV-III cell line retains its cytopathic activity against fresh normal human lymphocytes (unpublished data). Using these clones as probes, we also detected HTLV-III viral sequences in infected cell lines other than H9/HTLV-III that were established from different AIDS patients, and in fresh uncultured lymphoid tissues of AIDS patients¹⁹. These findings suggest that the cloned HTLV-III genomes reported here represent the probable aetiological viral agent of AIDS. The finding of two variant forms of HTLV-III in the H9/HTLV-III cell line could reflect cumulative *in vitro* mutations in a highly replicative virus. The two forms could also represent different isolates as, when first established, the H9/HTLV-III cell line was infected with pooled material from several different AIDS patients². Preliminary studies of other HTLV-III isolates indeed indicate that HTLV-III, unlike HTLV-I and HTLV-II, exhibits substantial diversity in its restriction enzyme cleavage pattern when isolates from different patients are compared¹⁹. Further characterization and sequence analysis will help to define the natural variability of this virus, which has important implications with respect to its pathogenicity and origin, and attempts at preventive measures for AIDS. The availability of the cloned HTLV-III genome should also now allow direct comparison of this virus with a similar group of retroviruses described by other investigators⁸⁻¹¹ which has also been linked to the pathogenesis of AIDS and which appears to be immunologically and morphologically indistinguishable from HTLV-III (M. Sarngadharan *et al.*, unpublished). Finally, the demonstration of a substantial amount of unintegrated viral DNA in the chronically infected cell line H9/HTLV-III, distinguishes HTLV-III from HTLV-I, HTLV-II and most other retroviruses. It will be important to determine whether the presence of unintegrated DNA has a role in the cytopathicity of HTLV-III, as has been proposed for certain other retroviruses^{20,21}.

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Metabolic oxidation phenotypes as markers for susceptibility to lung cancer

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That bronchial carcinoma is not an inevitable consequence of cigarette smoking has stimulated the search for host factors that might influence the susceptibility of the individual smoker. One plausible host factor would be a polymorphic gene controlling the metabolic oxidative activation of chemical carcinogens, giving rise to wide inter-subject variation in the generation of cancer-inducing and/or promoting species. Recently, three genetic polymorphisms of human metabolic oxidation have been demonstrated (as characterized by debrisoquine, mephenytoin and carbocysteine), with the metabolism of several substrates exhibiting the phenomenon¹⁻³. Debrisoquine 4-hydroxylation segregates into two human phenotypes, each comprising characteristic metabolic capability⁴⁻⁶. We report here the frequency of debrisoquine 4-hydroxylation phenotypes in age-, sex- and smoking history-matched bronchial carcinoma and control patients. Cancer patients showed a preponderance of probable homozygous dominant extensive metabolizers (78.8%) with few recessive poor metabolizers (1.6%) compared with smoking controls (27.8% and 9.0% respectively). We conclude that the gene controlling debrisoquine 4-hydroxylation may be a host genetic determinant of susceptibility to lung cancer in smokers and that it represents a marker to assist in assessing individual risk.

The metabolism of debrisoquine was examined in 479 cigarette smokers who had or had not presented with bronchogenic carcinoma, in order to determine the frequency of extensive metabolizer (EM) and poor metabolizer (PM) phenotypes in each group. Patients were recruited from areas of London within the Islington District, Bloomsbury District and Wandsworth District Health Authorities and were admitted primarily to Chest Unit beds at Whittington Hospital. All were white Europeans with a positive history of cigarette smoking (>20 pack-yr, that is, number of packs of 20 cigarettes per day × number of years of smoking). Subjects were excluded if chemotherapy or drugs known to interfere with the phenotyping test¹ had been given, if there were signs of abnormal hepatic or renal function and if additional acute conditions such as heart failure or severe chest infection obtained. The cancer patients ($n=245$) had a definite diagnosis of bronchogenic carcinoma proven by histology (108), cytology (85) or histology/cytology (44) from samples obtained at bronchoscopy (194), transcutaneous needle biopsy (24), mediastinoscopy (9) and pleural biopsy (6). Cell types comprised squamous cell (138), small cell (68), large cell (8) and undifferentiated (1) carcinomas, together with 30 adenocarcinoma patients. Control patients ($n=234$) were smokers with chronic airflow limitation, without evidence of carcinoma. Each patient received no drugs after 21.30 h the day before the test, nor for 2 h after the start of the test at 07.00 h. They were each given a 10 mg debrisoquine tablet orally; all urine was collected for the subsequent 8 h and analysed for its content of debrisoquine (D) and 4-hydroxydebrisoquine (4-HD) by electron-capture gas chromatography⁷. The metabolic ratio (urinary D/4-HD) thus determined was used to assign phenotype (EM, 0.1-12.6; PM, 12.7-100)⁵. Routine clinical chemistry and haematology were performed on a blood sample from each patient within 2 days before or after the test.

Cancer and control patients were similar in age (66.5 ± 7.4 (s.d.) and 67.2 ± 3.3 yr respectively), sex ratio (M/F) (1.82, 1.89) and smoking history (60.3 ± 24.0 , 59.4 ± 21.1 pack-yr). The results showed that the patients also had similar levels of plasma Na^+ (137 ± 6 , 137 ± 4 mM in cancer and control patients, respectively), HCO_3^- (27.0 ± 5.4 , 26.8 ± 4.9 mM), urea (5.0 ± 1.3 , $5.1 \pm$

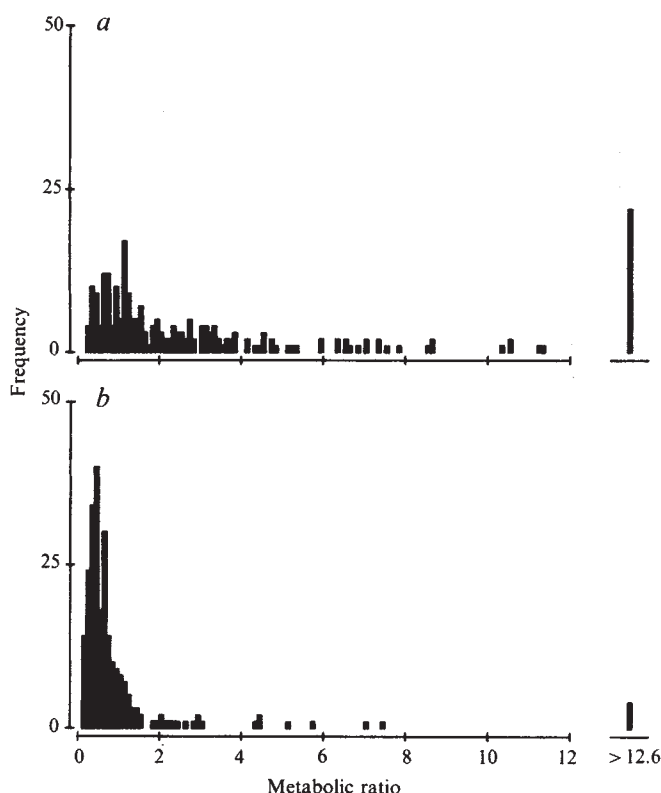


Fig. 1 Distribution of metabolic ratios (urinary D/4-HD) in smoking controls (a, $n = 234$) and bronchogenic carcinoma patients (b, $n = 245$).

1.6 mM), total protein (70.5 ± 4.8 , 70.9 ± 5.0 g l⁻¹ and bilirubin (8.6 ± 8.7 , 8.0 ± 7.6 μ M), and comparable white cell counts (9.0 ± 3.2 , $8.7 \pm 2.5 \times 10^9$ l⁻¹), mean corpuscular haemoglobin concentration (32.7 ± 2.2 , 32.8 ± 2.0 g per 100 ml) and platelet count (321 ± 85 , $325 \pm 89 \times 10^9$ l⁻¹). Values for plasma K⁺, albumin, alkaline phosphatase, aspartate aminotransferase, haemoglobin, packed cell volume, mean corpuscular volume and mean corpuscular haemoglobin all showed small but statistically significant differences between cohorts, but nevertheless fell within the 'normal' clinical range.

Debrisoquine 4-hydroxylation, however, showed several major differences between cancer and control cohorts (Fig. 1). While the metabolic ratios for 'smoking' controls were distributed across the full range of values and included 21 recessives (values > 12.6), the metabolic ratios for cancer patients were aggregated towards the left end (rapid metabolism) of the spectrum, with only four recessives. Figure 2 shows the combined data in logarithmic form, which both compresses and normalizes the distribution. Analysis of family pedigrees and a similar distribution for a healthy white British population⁶ has allowed estimation of the mean ($\pm$ s.d.) log₁₀ metabolic ratios for homozygous EM, heterozygous EM and recessive PM genotypes as -0.26 ± 0.23 , 0.13 ± 0.33 and 1.44 ± 0.31 , respectively. Remarkably, the additive cancer and control distributions have major modes at -0.35 , 0.05 and 1.75 , which almost certainly correspond to the three genotypes (Fig. 2). Thus, most cancer patients with log₁₀ metabolic ratios of less than 0 (193/245; 78.8%) probably have homozygous dominant genotypes, whereas the majority of smoking controls (148/234; 63.2%) with log₁₀ values between 0 and 1.1 (Fig. 2; ratios 1–12.6 in Fig. 1) are probably heterozygotes. Probable homozygous individuals in the control group were 27.8% (65/234) EM and 9.0% (21/234) PM genotypes. Finally, inspection of the extremities of the distribution reveals not only that there are no controls with ratios of 0.1–0.2, but also that the PM mode is almost devoid of cancer patients. Of the four cancer patients with a PM phenotype, two were adenocarcinoma patients and one had a 150 pack-yr cigarette consumption.

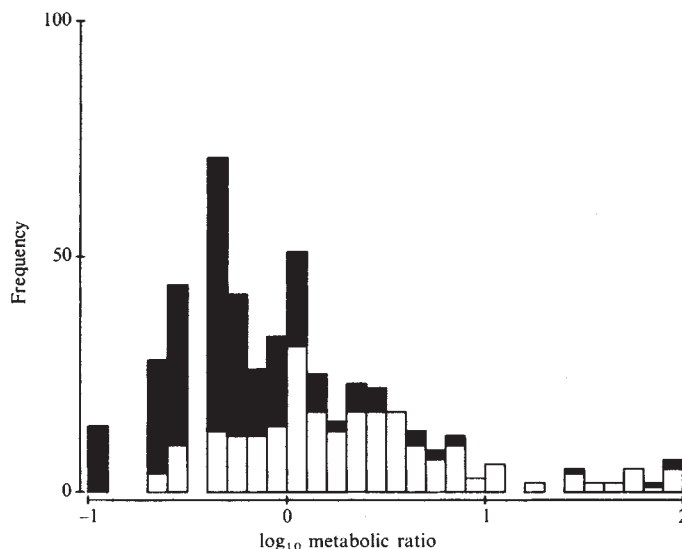


Fig. 2 Distribution of log₁₀ metabolic ratios for cancer (solid bars) and control (open bars) groups combined.

Care has been taken to ensure similarity between the two cohorts for factors which could influence either the disease (age, sex, smoking history) or the phenotyping test (hepatic/renal impairment, ethnicity, recent drug history). Nevertheless, the question arises as to whether or not the metabolic differences between cohorts which we report here contribute to the cause of lung cancer or are a result of it. Several reasons support the former interpretation of these data. First, our own findings in patients with cancer at other sites such as colon, rectum and urinary bladder, suggest that the presence of a tumour has no effect on the phenotyping test. Also, the debrisoquine metabolic ratio arises from hepatic metabolism of the administered drug and therefore major pathological changes within the liver might be expected to perturb the phenotyping test more than any other disease state. Appropriate data from a population of alcoholics with liver diseases of varying severity show that this is not the case⁸.

We propose, therefore, that the metabolic and genetic differences between white European cigarette smokers in this study who developed lung cancer by, on average, their mid-sixties and those who did not, reflect the aetiology of the disease. Homozygous dominants appear to be at high risk of developing cancer, whereas heterozygotes and recessives are at relatively low risk.

Debrisoquine 4-hydroxylase may not be involved in chemical carcinogen metabolism, but its gene may flank others with oncogenic potential, recessives for which will almost certainly be recessives for debrisoquine 4-hydroxylation. Such genes might be structural or regulatory genes for cytochromes P450, such as the *Ah* locus⁹, or indeed genes not involved in chemical carcinogen metabolism, for example, cellular oncogenes. We conclude that the metabolic oxidation phenotypes serve as genetic markers for differential lung cancer susceptibility.

This work was supported by grants from the Cancer Research Campaign. J.R.I. is a Wellcome Trust Senior Lecturer. We thank Nick Oates for computer analysis of the data. We also thank the consultants at St James Hospital, Balham and The National Temperance Hospital who allowed us to study their patients.

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Alu sequences are processed 7SL RNA genes

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7SL RNA is an abundant cytoplasmic RNA which functions in protein secretion as a component of the signal recognition particle¹. *Alu* sequences are the most abundant family of human and rodent middle repetitive DNA sequences (reviewed in ref. 2). The primary structure of human 7SL RNA consists of an *Alu* sequence interrupted by a 155-base pair (bp) sequence that is unique to 7SL RNA³. In order to obtain information about the evolution of the *Alu* domain of 7SL RNA, we have determined the nucleotide sequence of a cDNA copy of *Xenopus laevis* 7SL RNA and of the 7SL RNA gene of *Drosophila melanogaster*. We find that the *Xenopus* sequence is 87% homologous with its human counterpart and the *Drosophila* 7SL RNA is 64% homologous to both the human and amphibian molecules. Despite the evolutionary distance between the species, significant blocks of homology to both the *Alu* and 7SL-specific portions of mammalian 7SL RNA can be found in the insect sequence. These results clearly demonstrate that the *Alu* sequence in 7SL RNA appeared in evolution before the mammalian radiation. We suggest that mammalian *Alu* sequences were derived from 7SL RNA (or DNA) by a deletion of the central 7SL-specific sequence, and are therefore processed 7SL RNA genes.

The signal recognition particle (SRP) mediates the translocation of secretory proteins across the endoplasmic reticulum⁴⁻⁶. SRP consists of six different polypeptides and one molecule of 7SL RNA¹. Previous studies have shown that 7SL RNA is an essential component for SRP function¹.

In the human genome, *Alu* DNA constitutes the dominant family of short middle-repetitive mobile sequences⁷, and in addition, *Alu* equivalents have been described in the genomes of other primates^{8,9}, as well as in rodents^{10,11}. Human 7SL RNA is an abundant cytoplasmic RNA of 300 nucleotides which was shown to be partially homologous with *Alu* DNA¹². Approximately 100 nucleotides at the 5' end and 45 nucleotides at the 3' end of the RNA are homologous with the human *Alu* right monomer consensus sequence⁵. The central portion of 155 nucleotides shows no homology with *Alu* DNA and is unique to 7SL RNA (Fig. 1). The entire human and rodent *Alu* sequences are represented in the 7SL molecule (see Fig. 1).

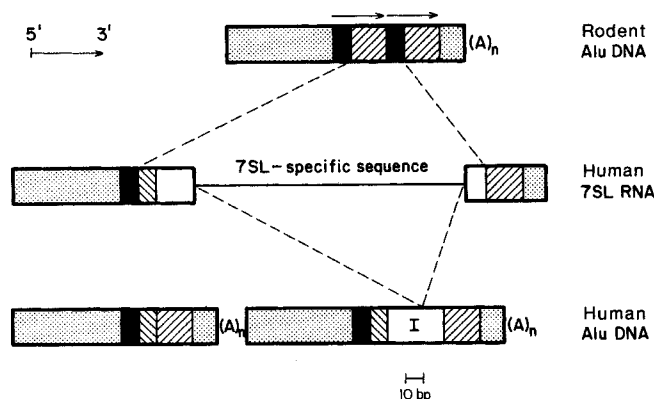
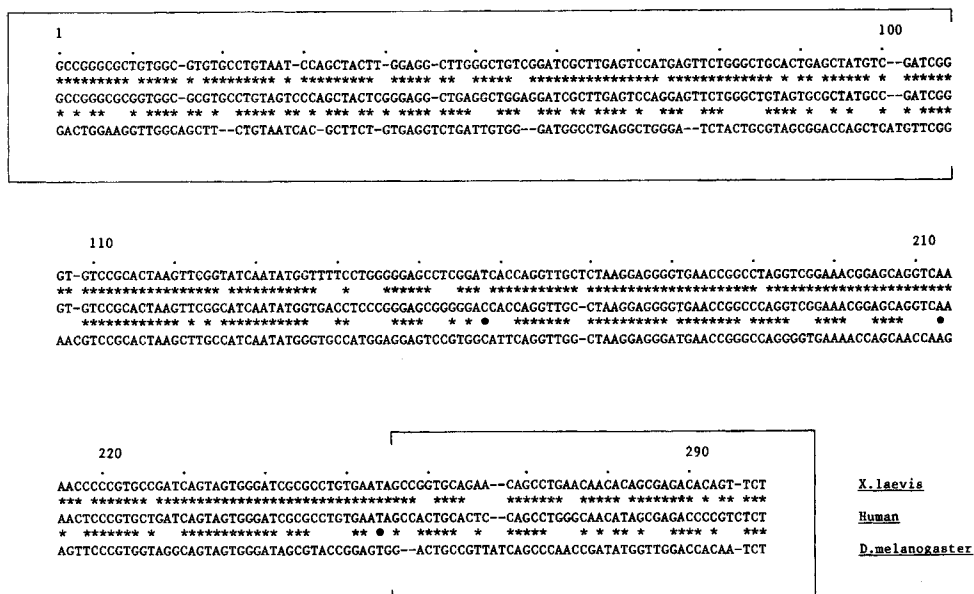


Fig. 1 The structural relationship of human 7SL RNA to the consensus sequences of human and rodent *Alu* DNA. Homologous sequences are indicated by identical shading. Human *Alu* DNA is a head to tail dimer of two similar sequences ~130 bp long. The right monomer contains an insert (I) which is not present in the left half²³. The rodent *Alu* equivalent sequence is a monomer^{10,11}. We have found that the mouse B1 *Alu*-equivalent consensus sequence compiled by Kalb *et al.*²⁴ contains an internal tandem duplication of 30 bp between positions 62 and 121. The type I Chinese hamster *Alu*-equivalent sequence¹¹ has a similar structural arrangement. Arrows above the rodent *Alu* DNA indicate the position of the 30-bp tandem duplication; (A)_n denotes an A-rich sequence which follows the *Alu* sequence at the 3' end.

To obtain information about the evolution of 7SL RNA and its possible relationship to *Alu* sequences, we have analysed the 7SL sequence in *X. laevis* and *D. melanogaster*. A cDNA copy of *in vitro* polyadenylated 7SL RNA of *X. laevis* was prepared as described previously³. For *Drosophila*, a bacteriophage library of embryonic DNA was screened for sequences homologous to the 155-nucleotide portion of human 7SL RNA not homologous with *Alu* DNA, using the 125-base pair (bp) *Sau*3A fragment of clone p7L1 as a probe³. Restriction endonuclease mapping of three independent positive phage clones and subsequent sequence determination of appropriate DNA fragments established that there are two identical genes coding for 7SL RNA in *D. melanogaster* (C.T. and Jean Burkhardt, manuscript in preparation). Figure 2 compares the primary structures of the *Xenopus* and *Drosophila* 7SL DNA with the human counterpart. The length of the 7SL RNAs of *Xenopus* and *Drosophila* is comparable with that of human 7SL RNA, which agrees with the observation that the three 7SL RNAs have the same electrophoretic mobility¹³. However, we have not determined the

Fig. 2 The nucleotide sequences of *Xenopus* and *Drosophila* 7SL DNA and their comparison with the human 7SL cDNA. The nucleotide sequence of a cDNA copy of *X. laevis* 7SL RNA (top line) and of the *D. melanogaster* 7SL RNA gene (bottom line) were determined by the chemical degradation²⁵ and the dideoxy-chain termination methods²⁶. The anticoding strand of both 7SL DNAs was aligned with the sequence of human cDNA clone 7L1 (ref. 3). The sequences are numbered with respect to the first nucleotide of the human 7SL cDNA. The first four nucleotides of cDNA clone 7L1³ are not included, because they are not represented in human 7SL RNA (E.U., unpublished observation). Asterisks denote homology between human 7L1 DNA and the amphibian or insect molecule, respectively. Dashes are occasionally introduced to maximize homology. The *Alu*-like portions of the human 7SL DNA are boxed.



precise 5' and 3' ends of *Xenopus* and *Drosophila* 7SL RNA. The *Xenopus* sequence is strikingly homologous to the human one over its entire length. There is only a 13% divergence and the mismatches are distributed along the entire length of the molecule. As these two 7SL DNAs are extremely similar, the appearance of the *Alu*-like sequence in the 7SL RNA molecule evidently preceded the mammalian radiation in evolution.

When the sequence of the *Drosophila* 7SL DNA is compared with its human and amphibian counterparts, there is an ~64% homology; however, extended regions in the central 7SL-specific portion can be seen, where the sequences have been almost perfectly conserved. These 7SL-specific homologies may reflect a strong functional constraint acting on these sequences. The *Alu*-like portions of the human and insect 7SL DNA can be only poorly aligned. Nonetheless, the vestiges of the mammalian *Alu*-like sequence can be recognized in the *Drosophila* 7SL DNA. These results, taken together, strongly suggest that the evolution of the *Alu*-like portions of 7SL RNA has taken place gradually. No dramatic event such as the insertion of the 155-bp 7SL-specific DNA into a human *Alu* sequence, as originally suggested³, can account for the identical composite structure of 7SL RNA in two lower organisms. If 7SL RNA genes were in fact assembled from two different types of sequences, such an event must have occurred before the appearance of insects.

Because the structure of 7SL RNA has been extensively conserved in evolution, we propose that 7SL RNA genes represent the ancestor of the *Alu* sequence and that *Alu* DNA arose from the 7SL RNA information through a deletion of the central 7SL-specific sequence. Such a deletion could have occurred either at the DNA level (through non-homologous recombination within or between 7SL RNA genes) or at the RNA level (due perhaps to an aberrant RNA joining or splicing event).

The discovery of 'processed genes' which lack intervening sequences and closely resemble the mature mRNA structure¹⁴ and the finding of pseudogenes for the small nuclear RNAs¹⁵ have provided evidence that RNA information can flow back into the genome. We speculate that the prototypical *Alu* sequence is a processed 7SL RNA gene. It has been observed that the 7SL RNA molecule within SRP is very resistant to micrococcal nuclease digestion^{1,16}. Interestingly, only three major discrete RNA fragments are generated by limited nuclease digestion of SRP. Two fragments of 72 and 45 nucleotides span the 5' and 3' *Alu*-like portions, respectively, while the third product of nuclease cleavage spans the entire 7SL-specific sequence of the RNA¹⁶. These experiments clearly indicate that an enzyme probe can readily recognize the boundaries of the *Alu*-like and 7SL-specific domains of 7SL RNA in the intact ribonucleoprotein particle. This finding suggests that SRP itself may contain the RNA in a conformation which promotes excision of the 7SL-specific domain. Once this happens, the 5' and 3' *Alu*-like portions could be ligated together, generating a contiguous *Alu* RNA. The sequence of the spliced RNA could then have been re-integrated into the genome.

Several groups have proposed that processed genes could arise from the reverse transcription of cellular RNA species, followed by integration of the cDNA into new chromosomal sites in germ-line DNA^{14,17-20}. In this context, 7SL RNA was first discovered as a component of avian and murine retroviral particles²¹. Because retroviruses encode a reverse transcriptase and carry the enzyme within the virion, it is tempting to speculate that retroviruses are responsible for the generation of an *Alu* sequence from 7SL RNA.

Using the homologous 7SL DNAs as probes, we have searched in the genomes of *Drosophila* and *Xenopus* for 7SL RNA-related sequences (data not shown). We note that the *Drosophila* genome contains only two 7SL RNA genes and no other cross-hybridizing sequences. In contrast, *Xenopus* DNA is rich in sequences capable of cross-hybridizing to the homologous 7SL DNA probe. Some of these sequences might represent *Alu*-like sequences, as suggested by previous studies²². It is possible that the excision of the *Alu* sequence from 7SL RNA (or DNA) may have occurred several times in evolution. Once freed of 7SL

sequences, *Alu* DNA may no longer be subject to the functional constraints which operate on the 7SL RNA genes and thus may be capable of evolving independently from 7SL RNA.

We thank Jean Burckhardt for making the *Drosophila* 7SL recombinant clones available to us, Alan Weiner and Frank Richards for advice and support, Peter Walter for his continuous interest, Alex Young for technical assistance and Manny Ares, Joan Steitz, Peter Walter and Alan Weiner for critical reading of the manuscript. E.U. was the recipient of a long-term EMBO fellowship and is on leave of absence from the Istituto di Biologia Generale, Università di Roma. This work was supported in part by NIH grants GM 31073 and GM 31335 to Alan Weiner and AI 08614 to Frank Richards.

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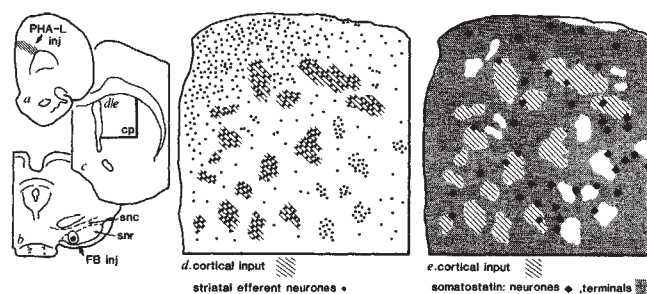
Erratum

The neostriatal mosaic: compartmentalization of corticostriatal input and striatonigral output systems

C. R. Gerfen

Nature **311**, 461-464 (1984)

THE key was omitted from Fig. 1. A correct version appears below:



History peopled

W.F. Bynum

The Discoverers: A History of Man's Search to Know His World and Himself.

By Daniel J. Boorstin.

Dent/Random House: 1984. Pp. 768. £15, \$25.

DANIEL Boorstin has been Librarian of Congress since 1975 and his previous dozen books (including one Pulitzer Prize winner) have been on American history. *The Discoverers* is his homage to the rest of the world. America and Americans are of course part of his story, but only a relatively small one. His primary focus is Europe and Asia and those who have contributed to "man's search to know his world and himself".

The book's sub-title and size suggest that Mr Boorstin has set himself a task on the grand scale. A look at the volume's four main sections confirms this: "Time"; "The Earth and the Seas"; "Nature"; "Society". This organization provides a rough chronological thrust to his narrative. "Time" deals with ancient calendars, seasonal reckoning and time-telling from antiquity to the coming of clocks and watches. John Harrison's chronometer for calculating longitude links that section with the next, which examines cosmology and cartography, navigation and exploration, from long before Ptolemy to long after Columbus. The section on Nature takes off from the Scientific Revolution, when the telescope and microscope extended the range of vision, and makes its leisurely way through some of the principal scientific disciplines of the eighteenth and nineteenth centuries, such as taxonomy, palaeontology and evolutionary biology. Finally, "Society" describes a fascinating mélange of topics, from printing and books to the writing of history, the discovery of Troy and Keynesian economics.

The result is both less and more than a conventional history of science. It is less because the coverage is sometimes patchy, particularly of the physical sciences after Newton. There is virtually nothing on chemistry (Lavoisier rates only one passing mention) and two centuries of physics are covered in a rather unfortunate closing chapter (part of "Society", not "Nature") in which Mr Boorstin leaves himself only a single page to dispense with James Clerk Maxwell, J.J. Thomson, Ernest Rutherford and Albert Einstein. Seventeenth-century microscopists such as Leeuwenhoek and Malpighi get full treatment, but there is nothing on cell theory of the nineteenth century. Galen, Vesalius, Paracelsus and Harvey (mistakenly knighted on p. 459) get their due, but virtually no doctors after them except for Freud.

Mr Boorstin has therefore not given his

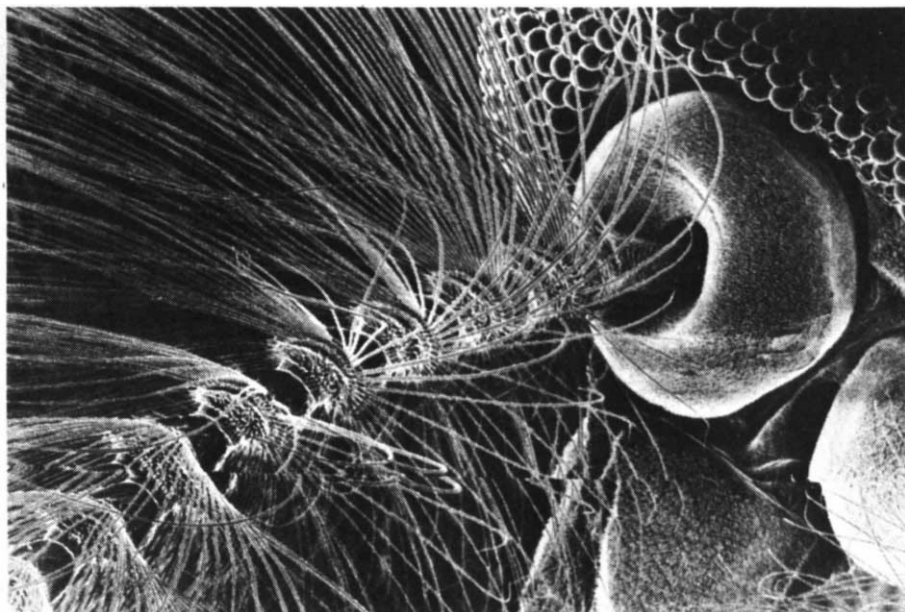
readers much that a single-volume survey of the history of science and scientific discovery should; but he has more than compensated for this lacuna by offering a great deal more. Scattered through this magnificent volume are unexpected themes and people treated with what can only be described as affectionate authority. The Jesuits and their clocks in China; Islamic attitudes to the printing press; the growth of public libraries; the discovery of "prehistory": these and dozens of other vignettes share centre stage with a multinational cast of unfamiliar characters, such as Sir Anthony Panizzi, Italian-born librarian of the British Museum; Martin Waldseemüller, the German clergyman who named America; Chêng Ho, the eunuch Chinese navigator; and Lorenzo Valla, the Italian theological scholar. If the book contains many curious and delightful byways, seekers of more familiar discoveries and discoverers will not be disappointed, for individuals such as Copernicus and Galileo, Henry the Navigator and James Cook, Linnaeus and Darwin, are sympathetically covered. The level of accuracy throughout is unusually high in a book which covers such a vast terrain. A 30-page bibliographical essay guides the reader to the range of sources from which material has been drawn.

Mr Boorstin seems particularly to enjoy biography, and most of the book's 82

chapters are arranged around the achievements of two or three relevant people. This penchant for biography can be seen by the frequency with which he uses biographical metaphors and similes: Karl Marx was "a latter-day Paracelsus", Paracelsus in turn was "a medical Don Quixote". Newton was "the Galahad of the Scientific Quest", whereas several individuals (John Dalton, for example) were Columbooses. Boorstin has a fine eye for anecdote, is particularly good on word derivations (among them "punctual", "travel", "hour", "Bible", "culture") and writes always with elegance and zest. The volume is studded with memorable phrases and epigrams. One such epigram, not directly from his pen but quoted from Callimachus (305–240 BC), head librarian in Ptolemaic Alexandria, declares that "A big book is a big nuisance". If this is true, *The Discoverers* is the exception that proves the rule, for, like the Bible, this is a Good Book. It is best read slowly and in small doses, in order to savour the richness of the story. It is also best read with an historical atlas nearby, for despite the importance of geographical exploration among Boorstin's themes, there are no maps provided. Astronomical diagrams would also have helped readers grasp the differences between the cosmologies of the Babylonians, the Indians, Ptolemy and Kepler.

This book had its origins, Mr Boorstin tells us, in his first visit to Florence a half-century ago, and has been 15 years in the specific researching and writing. This long gestation has produced a splendid brain-child, a wise and optimistic volume which enlightens and entertains. Read it. □

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Seeing sense — base of the antenna of a male mosquito, photographed by Lennart Nilsson. The picture is reproduced from *Nature Magnified*, a collection of Nilsson's work published by Macdonald price £9.95.

Sprung from chains

Struther Arnott

Principles of Nucleic Acid Structure.

By Wolfram Saenger.

Springer-Verlag: 1984. Pp. 556. Pbk DM 79, \$29.50.

FROM time to time one reads a book or an account of an experiment and wishes, with more pleasure than envy, that one had produced it oneself. Wolfram Saenger's book is such a virtuosic production: well-written, profusely illustrated and astonishingly comprehensive. In it, fact and hypothesis are distinguished with rare scrupulousness. Any serious student or researcher will find it a mine of information. The merely curious now have a guidebook with which safely to explore the labyrinth of DNA and RNA structural studies.

Particularly praiseworthy is the way Saenger has managed to deal thoroughly but interestingly with all the fundamental business of conformational nomenclature and crystallographic esoterica. He has not failed to explore the many structural hypotheses still twisting slowly in the wind, but almost nowhere does he confuse these with experimentally well-founded models.

About a third of the book is devoted to exploring the nature and provenance of the polymorphism of DNA and RNA. Any polymer constructed with residues as complex as nucleotides should be extremely polymorphic. Even when restrained in plectonemically wound duplexes, with bases paired and stacked, polynucleotides have many degrees of structural freedom. Yet polymorphism in DNA and RNA structures has been persistently ignored or belittled, as a brief glance at history will show.

Astbury's early fibres were mixtures of A-DNA and B-DNA, but he interpreted their X-ray patterns in terms of a single allomorph with the residue repeat of B-DNA (0.34 nm) and the structural repeat of A-DNA (2.80 nm). Then, during the renaissance of X-ray studies in the early 1950s, Franklin and Gosling established that DNA was at least dimorphic. Provocatively, they named their newer form A, and the form worked on earlier by Wilkins, B. During the long process of reconciling the Watson-Crick hypothesis with a tangible structure, attention was focused on the more hydrated B form, and A-DNA was forgotten until the 1960s when RNA duplexes were discovered to have A-like structures. Despite conformational similarities the A structures are quite polymorphic, with helix pitches ranging from 2.8 nm to 3.9 nm and major grooves of strikingly variable widths. In the eponymous A-DNA this groove is clenched shut, excluding even water molecules, while in the A duplexes of largest pitch the

major groove is wide enough to accommodate a third polynucleotide chain. B-DNAs have been shown to be just as polymorphic with pitches ranging from 2.4 nm to 3.6 nm.

With Polonius-like retrospective wisdom one would expect the apotheosis of A and B polymorphism to be heteronomous duplexes in which one chain carries C3'-endo-puckered deoxyribose rings like the A structures and the other carries C2'-endo-puckered rings like B. The synthetic duplex poly d(A). poly d(T) indeed has such a structure. (Sadly this particular allomorph arrived too late to catch the Saenger omnibus.) Heteronomous duplexes, unlike allomorphs with identical chains, are highly polar. Perhaps bidirectional sequences on DNA in chromosomes function as valves or direction markers.

Clearly the great variety of (right-handed) helical structures trapped in fibres before 1980 implies at least as great a variability in DNAs and RNAs in other milieux. Saenger's book certainly exposes us to variety in the catalogue of static structures, but the lively world of nucleic acids, rich in movement, bending, breathing, vibrating and shimmering is largely unexplored. Perhaps the *frisson* of the discovery of left-handed DNA will emphasize the reality of polynucleotide variety and variability — but perhaps not!

The possibility of left-handed polynucleotide duplexes was recognized early on: Maurice Wilkins explored sinister

models of A- and B-DNA in the 1950s; later, others touted left-handedness for the D form of poly d(IC).poly d(IC), in hope, but erroneously. When a left-handed (Z) allomorph was eventually observed, ironically it indeed needed alternating purine-pyrimidine sequences. Z-like conformations have been observed with d(GC)_n.d(GC)_n, d(AC)_n.d(GT)_n and d(As⁴T)_n.d(As⁴T)_n but not yet with d(IC)_n.d(IC)_n — nor with d(AT)_n.d(AT)_n.

Depressingly, DNA is again being discussed in dimorphic terms; however now it is B and Z, not A and B! But Z-DNA is polymorphic too; relatively underwound duplexes with the peculiar dinucleotide motif of Z-DNA exist with residue rotations of 0°, -25°, -51° as well as the -60° of the first left-handed structure.

Current X-ray structural studies focus on oligonucleotides in single crystals and in poly(oligonucleotides) in fibres. These structures are less regular. Whether or not the excursions from regularity are related to base sequences is a lively issue. More ambitiously — and perhaps more wisely — there are increasingly successful studies of DNA bound to histones and other proteins which will reveal specifically how variety and variability in the genetic material can be exploited biologically. Among all its other riches, Saenger's book makes us aware of these too. □

Struther Arnott is Vice President for Research and Dean of the Graduate School at Purdue University, Indiana.

Magnetic mixture

D. Walton

Rock and Mineral Magnetism.

By W. O'Reilly.

Blackie/Chapman & Hall: 1984. Pp.220. £19.50, \$45.

A number of texts on rock magnetism have appeared over the past 20 years, but there is still a clear need for a solid, carefully written book on the subject. Sadly, this contribution does not fit the bill.

In general, the objective of books of this nature is to provide geophysicists with the information to be able to understand how the magnetic vector is produced in their samples. To do this it is essential first to understand the mineralogy, that is, the chemical and physical properties of the magnetic minerals and how these can change with temperature, pressure and chemical environment. Second, it is necessary to understand the physics — how the magnetic moments of individual grains arise, and how assemblies of such grains produce the resultant magnetic vector of the sample given the conditions of temperature, magnetic field, cooling rate and so on to which the sample was exposed.

Dr O'Reilly certainly meets the first of

these goals. There is a wealth of useful information on the mineralogy of magnetic minerals; and while it is quite difficult to find the desired data, the fairly complete index mitigates the problem to a great extent.

Just as certainly, however, the book falls short of the second requirement: the mathematics is quite inadequate, with formulae simply being presented with no derivation and often supported by no reference. The equations are not numbered, often they are not displayed, simply appearing in the body of the text. Furthermore, the author is not only selective in his choice of references (perhaps understandably), but much of the literature he has opted to quote is oversimplified to such an extent that it is wrong. For example only one of Neel's famous papers is referred to, although these contain the basic physics and have not been superseded.

Neel's papers still form the basis for all rock magnetic theory. In discussing the physics of the subject, authors of textbooks would be well advised to begin with them. And if they intend to provide an accurate and reliable source of information for the uninitiated, it might be safer to end with them. □

D. Walton is Professor of Physics at McMaster University, Hamilton, Ontario.

Making a meal of primate ecology

R.D. Martin

Adaptations for Foraging in Nonhuman Primates: Contributions to an Organismal Biology of Prosimians, Monkeys, and Apes.

Edited by Peter S. Rodman and John G.H. Cant.

Columbia University Press: 1984. Pp.351. Hbk \$45.50; pbk \$18.50.

FOR sound ecological reasons, field-studies of non-human primates have increasingly focused on quantitative studies of feeding behaviour (taken in the widest sense). Food provides the energy required for all activities, but collection incurs a cost, particularly in terms of locomotor energy expenditure, and the overall cost-benefit equation is clearly of central importance in our understanding of the behavioural ecology of any species. That equation, however, contains a large number of different variables and thus a wide-ranging approach to it is necessary.

Adaptations for Foraging in Nonhuman Primates, a collection of 11 chapters edited by Peter Rodman and John Cant, brings together accounts of a variety of different aspects of primate feeding adaptations with this end in mind. The book is, in fact, the outcome of a special symposium on primate "foraging" held at the annual meeting of the American Association of Physical Anthropologists in 1980.

Rodman and Cant have taken an extremely broad view of primate feeding adaptations, including a great number of morphological, physiological and behavioural components. Clearly, an integrated approach to all of these components is much to be desired. This is certainly true with respect to evolutionary biology, and it is gradually being recognized that it is also likely to apply to behavioural ecology.

To take just one example, abundantly illustrated in the book: Kleiber's Law governing the scaling of basal metabolic rate has far-reaching implications for morphology, physiology and behaviour, because of its direct relevance to energy budgets. Complex feedback relationships are involved and explanatory models are really inadequate without some appreciation of them. In leaf-eating primates, for instance, the overall energy budget depends not only on behavioural factors, but also on special adaptations in morphology (such as enlargement of part of the digestive tract to house a flourishing gut flora) and in physiology (such as possession of special physiological capacities for coping with plant secondary compounds). Indeed, it now seems likely that most or all leaf-eating primates exhibit relatively low basal metabolic rates, after body size has been taken into account, and this con-

stitutes part of their overall "strategy". Some of this interdependency is neatly illustrated in Milton's thoughtful chapter on the role of food-processing factors in primate food choice.

There is, of course, a problem involved in taking such a wide-ranging view of the subject. It is all very well being presented with series of tasty dishes; at the end of the meal, digestion must be feasible. In this respect, comparisons are bound to be made with *Primate Ecology*, edited by Tim Clutton-Brock and published by Academic Press in 1977, which very effectively brought together the results of a score of good, quantitative field studies of primate feeding behaviour, but which devoted little attention to morphology, physiology or locomotor behaviour.

Some of the chapters in the present volume are gems in their own right, either as useful syntheses or as accounts of new developments. Among the former, Kay's chapter on morphological correlates of feeding behaviour is especially noteworthy in bringing together several different lines of (predominantly dental) evidence that permit quite reliable inferences to be made about broad dietary habits from anatomical features. This has evident benefits for the interpretation of primate fossils, but the link with energy budgets of living primates is somewhat tenuous.

Of the contributions dealing with novel approaches, Milton's chapter centres on a broad, cross-species comparison of transit times for food material passing through the gut. This adds a new dimension to studies of gut parameters in relation to primate dietary habits. Two further chapters examine locomotion in specific relation to feeding behaviour; Crompton reports on a study of two bushbaby species in South Africa, while Garber describes a study of Geoffroy's tamarin in Panama. Both accounts are notable for their admirable emphasis on quantitative data and for their careful extraction of points for an explanatory framework. Although it is argued in other chapters that there is no clear relationship between diet and locomotor patterns across species, these two studies definitely demonstrate that locomotion is intimately related to food-gathering within species.

A number of authors here use comparisons of two species in order to investigate underlying relationships. The most suc-

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● *Geographical Ecology: Patterns in the Distribution of Species* by Robert H. MacArthur, which first appeared in 1972. Publisher is Princeton University Press, price is \$19.50.

● *God and the New Physics* by Paul Davies. In the United States the paperback edition is published under the imprint of Touchstone Books, in Britain as a Pelican. Price is \$7.95, £3.95. For review see *Nature* 305, 833 (1983).

● *The Evolution of Human Consciousness* by John H. Crook. Publisher is Oxford University Press (Clarendon). Price is £9.95.

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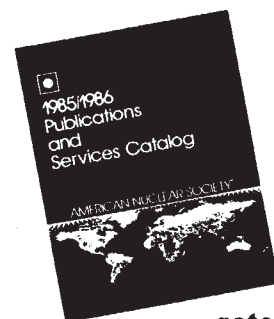
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cessful of them is Waser, who compares two mangabey species. His chapter is actually most remarkable for its careful documentation of the close similarity in behavioural ecology between the arboreal mangabey species (*Cercocebus albigena*) that he studied in Uganda and the largely terrestrial species (*C. galeritus*) studied by Katherine Homewood in Kenya. Waser attempts to relate the relatively limited differences between the two species to ecological differences, but it is somewhat difficult to test the inferences made. This problem is even more acute with other chapters based on a comparison between two species. For instance, Temerin, Wheatley and Rodman attempt to analyse the relationship between body size and foraging in primates by comparing *Macaca fascicularis* (the crab-eating macaque) with *Pongo pygmaeus* (the orang-utan). These two primates do, of course, differ in numerous ways, but a comparison between them cannot reliably reveal which of those differences is due to body size alone. (Curiously, the book includes no allometric analyses other than those contained in Kay's chapter; this technique has much to offer, especially in identifying body-size effects.)

Finally, special mention should be made of Post's essentially theoretical, but

nonetheless challenging chapter arguing that current optimal foraging models are generally designed for fine-grained habitat conditions and are therefore inadequate for interpretation of primate feeding behaviour in patchy habitats.

Perhaps the most surprising point about the book as a whole is that there is no explicit discussion of the crucial distinction between "foraging" (the search for food) and "feeding" (actual ingestion). These terms are very loosely used in the literature and a clearing-up exercise is long overdue. But the book's principal drawback resides in its lack of cohesiveness; in the event, the meal is pretty indigestible. The introduction by Rodman and Cant is interesting in a historical sense, but theoretically superficial, while the closing chapter by Cant and Temerin, which could have served to bring the key points together, actually dissolves into a diffuse listing of large numbers of potential interacting variables. So although *Adaptations for Foraging in Nonhuman Primates* contains much of value in terms of individual chapters, it falls far short of the synthetic view of primate feeding adaptations that should one day emerge. □

R.D. Martin is Professor of Physical Anthropology at University College London.

How (and whys) in molecular biology

Peter Little

Transcription and Translation: A Practical Approach.

Edited by B.D. Hames and S.J. Higgins.
IRL Press: 1984. Pp. 328. Pbk £12, \$24.

ONE of the problems of compiling a practical manual in molecular biology is to decide when to do it. The speed with which the subject progresses makes it difficult to judge when a method has progressed from research topic to established procedure. Perhaps the easiest way to identify areas that are ripe for such a treatment is to ask whether justification for use of the method has to be supplied with the protocol.

By this criterion, a number of the topics covered in Hames and Higgins's collection of articles were not ready for inclusion in a methods handbook. The point is most apparent in the first half of the book, which covers eukaryotic transcription and translation. Extensive protocols are provided in an annoying tabular form but they are heavily interspersed with results and discussion — this is particularly true of the chapter on expression of DNA in mammalian cells from Spandidos and Wilkie, who have otherwise written an interesting and extensive review of a rapidly developing field.

The second half of the book is devoted to

prokaryotic transcription-translation systems and eukaryotic *in vitro* methods and is a useful source of protocols, albeit in the same unsatisfactory format. Here Colman contributes a helpful and informative account of the use of *Xenopus* oocytes for translation of nucleic acids. Sandwiched between the two halves is a chapter on the use of *E. coli* polymerase to transcribe chromatin — a field which seems to me, even after reading this chapter, to have limited intellectual justification in view of extensive progress in *in vitro* techniques for faithful transcription of DNA by eukaryotic polymerases (well documented here by Manley).

It is worth comparing this volume with *Molecular Cloning: A Laboratory Manual*, published by Cold Spring Harbor Laboratory (reviewed in *Nature* 300, 782; 1982). The most striking difference is that in the latter book each method is presented clearly, without detailed discussion as to why one would want to make, for example, a phage lambda library. If the editors could have put together a manual on transcription and translation in a similar fashion, they would have produced a very worthwhile publication, but it is probably a few more years before this can be done. As it is the book contains several useful articles, but it is not one which I would buy for my laboratory. However, I think I would borrow it! □

Peter Little is a Staff Scientist at the Institute of Cancer Research, Chester Beatty Laboratories, London.

Piecing it together

John M. Charap

The Ideas of Particle Physics: An Introduction for Scientists.

By J.E. Dodd.

Cambridge University Press: 1984. Pp. 202. Hbk £22.50, \$44.50; pbk £8.95, \$18.

THE IDEAS of particle physics are probably no more difficult than those of many other sciences, but they are certainly less familiar. Notwithstanding the fact that the discoveries of recent years together represent one of the great achievements of modern science, most non-physicists would be hard pressed to explain why there is such confidence in the validity of the "standard model" of electroweak and QCD interaction between leptons and quarks, still less to point beyond present frontiers to more or less credible speculation.

There has long been a need for a book which would enable scientists from other disciplines to share more fully in the continuing endeavour of particle physicists, pursued on a grand scale and addressing the most fundamental question about the ultimate structure of matter; after all, the advances of the past few years have brought about as profound a revolution of our knowledge of the basic constituents of all matter and of their interactions as did the heroic work of Bohr and Rutherford for our understanding of the constitution of atoms. Dr Dodd has written just such a book.

The stage is set with a brief introduction to the key ideas of relativistic quantum field theory, and the drama is allowed to unfold in a broadly chronological sweep. The mathematical demands are modest. Equations are used to summarize or to illustrate rather than to advance arguments. Diagrams likewise are there to elucidate and not to baffle, and the ubiquitous Feynman diagrams need hold no terror. The fact that there are rules which relate them quantitatively to measurable observables is mentioned but no such calculations are undertaken. And the book is bang up to date; the evidence for the top quark from the CERN $p\bar{p}$ collider is the only major post-publication discovery omitted.

If you consider yourself to be reasonably numerate, and want to learn about quarks and gluons, asymptotic freedom and colour confinement, electroweak unification, and W and Z bosons, Dr Dodd's book is an excellent place to start. More important, if you want to know how the jig-saw puzzle seems to be falling into place, making sense of the plethora of particles, then this is the book to read. □

John M. Charap is Professor of Theoretical Physics and Dean of the Faculty of Science at Queen Mary College, University of London.

Cellular and other biologies

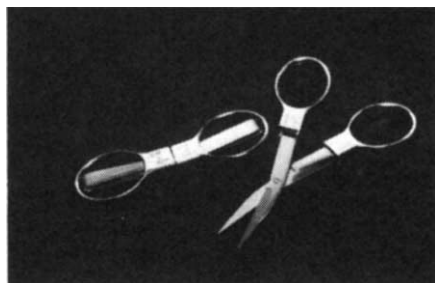
Many of the major laboratory equipment manufacturers are exhibiting at the current meeting of the American Society for Cell Biology.

● Newly introduced at the American Society for Cell Biology Meeting, the Dapple Systems, Inc. Video Image Measurement System (VIMS) performs direct on-screen measurement of features in video images produced by a camera mounted on a light or electron microscope or simply viewing a photograph. The video image and computer display are superimposed on a single monitor to allow marking of features. Measurement modes include outlining the periphery of features, tracing lengths, or making end points. Extensive statistical analysis programs report mean and variance, find comparisons between variables, and provide linear, log or polynomial fits. Distribution plots of one variable as a function of another are useful for studying correlations between two variables. The method of DeHoff and Rhines is employed to obtain distributions for the case of intersections of a planar surface through a solid with embedded features.

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● The Labconco RapidStill III is a compact automatic steam distillation unit used for nitrogen/protein determinations. Operation is completely automatic. After pressing the start button, the distillation process is controlled by a timer that determines the amount of water and sodium hydroxide used. One distillation takes less than 4 minutes. The distillation system automatically shuts down when the adjustable timer goes off.

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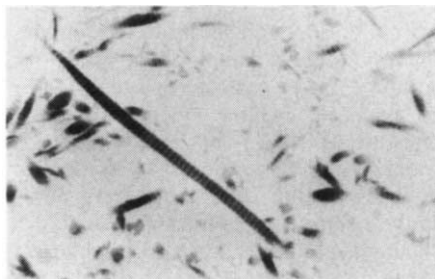
Fold-up scissors from B-J Scientific

● B-J Scientific Products of Albany, Oregon has introduced a compact, high-quality folding scissor called Slip-N-Snip, for use in the medical and scientific professions. These scissors are fabricated with stainless surgical steel blades and chrome-plated metal handles, and can actually cut through metal sheet with no damage to the blades. When not in use, Slip-N-Snip scissors can be easily foled up, with the blades completely shielded, and can be carried in a pocket with complete safety, unlike standard scissors.

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● RepliPlate is a new colony transfer pad for the rapid replica plating of bacterial colonies, from the BioProducts Department of Marine Colloids Division, FMC Corporation. Each pad is presterilized and ready-to-use. At least six precise replications can be made in a matter of seconds. RepliPlate pads simplify multiple-colony replication or library screening and are compatible with normal aseptic laboratory techniques. The pads are disposable to ensure biohazard containment and to prevent cross-examination.

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Electron micrograph of fibrillar collagen reconstituted from Vitrogen 100. Magnification $\times 30,000$.

● Vitrogen 100, the purified collagen for use in cell culture and biochemistry may now be purchased directly from Collagen Corporation of Palo Alto, California. Vitrogen 100 is a convenient, consistent and reproducible source of collagen which is economical to use. It is sterile, enzyme-solubilized bovine dermal collagen which has been extensively purified (99.9% pure), rigorously characterized and packaged for immediate use. Vitrogen 100 also eliminates problems with contaminants and batch-to-batch inconsistencies.

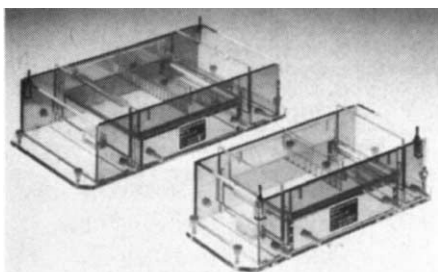
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● Pharmacia Fine Chemicals has introduced four new columns for high-performance preparative chromatography of proteins with sample loadings of up to 500 mg. The columns are based on the monodisperse ion exchangers Mono Q (anion exchanger) and Mono S (cation exchanger) with bed volumes of 8ml, Mono Q HR 10/10 and Mono S HR 10/10, or 20ml, Mono Q HR 16/10 and Mono S HR 16/10. Resolution is the same as is obtained in the well known columns, Mono Q HR 5/5 and Mono S HR 5/5, at sample loadings which are up to 20 times greater. Scale is direct with well described scaling factors which makes methods development straight forward and reliable. The columns are supplied for direct connection to the Pharmacia FPLC System. Accessories for hook-up to other HPLC systems are available.

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● ExpertVision, a new microcomputer-based system from Motion Analysis Corporation, allows the intricate movements of everything from microscopic organisms to human beings to robots to be analysed in detail. ExpertVision automatically converts images captured on videotape to a form that can be quickly analyzed by a computer. The system uses the video camera to capture images in real time. These analog video images are then played into a proprietary video processor that digitizes the image outline and inputs the data into a 16-bit, UNIX-based, high-resolution graphics workstation. The video processor displays the raw or processed images on the monitor, giving the operator immediate visual feedback. Once the digitized image is stored on the workstation's 42-megabyte hard disk, ExpertVision's software transforms the raw data into graphic images that depict the path of the motion. The digitized data are then analysed by a 16/32-bit microprocessor. The analysis can be displayed as numeric tabulated summaries, in graphic form (line graphs, bar charts, and three-dimensional images), or both. Hard-copy output of all screen displays can be generated on the system's printer/plotter.

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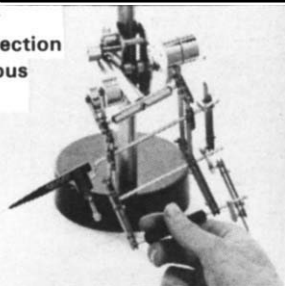
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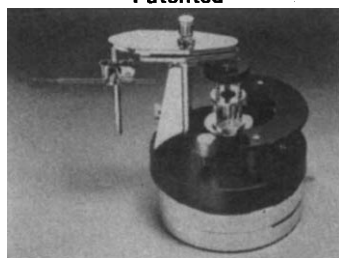
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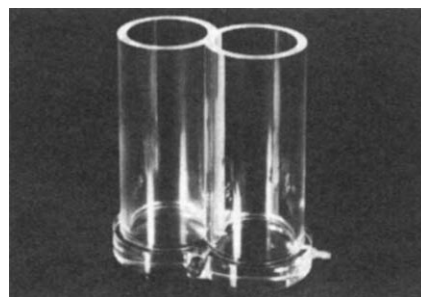
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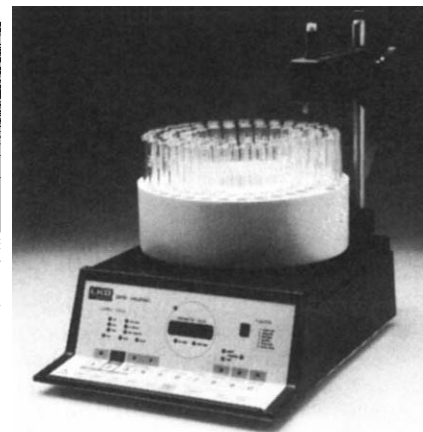
● Laboratory Computer Systems, Inc. has added statistical analysis software to its Micro-Plan II image analysis system. The software is suitable for use with the IBM PC or PC/XT. The Micro Plan II is a low-cost, tablet-oriented image analyser that performs measurements such as length, area, centre of area, form factor, feret $x-y$, and maximum diameter of two-dimensional features traced on the digitizing tablet. The software allows the Micro-Plan II user to analyse these measurements statistically and to create data files in special formats that can be used for publication or read by Lotus Development Corporation's spreadsheet program 1-2-3. **Circle No. 108 on Reader Service Card.**

● Cambridge Isotope Laboratories is now offering carbon-13 labelled standards for the higher chlorinated homologue of the dioxin series. The particular standards are 1,2,3,4,7-pentachlorodibenzodioxin, 1,2,3,4,7,8-hexachlorodibenzodioxin, 1,2,3,4,7,9-heptachlorodibenzodioxin and 1,2,3,4,6,7,8,9-octachlorodibenzodioxin. Each standard is completely labelled with carbon-13 at an enrichment level of 99% or greater giving a net mass spectrometric response of + 12 AMU. Each standard is supplied at a specific concentration in nonane solution. The unlabelled standards of these same isomers are also available in standard solutions. Stable isotope labelled standards are already available for 2,3,7,8-tetrachlorodibenzodioxin and 1,2,3,4-tetrachlorodibenzodioxin. The addition of the penta through octa standards now makes it possible to do accurate GC/MS analyses on the whole series of most important dioxin isomers. **Circle No. 109 on Reader Service Card.**



Hoefer's new gradient makers.

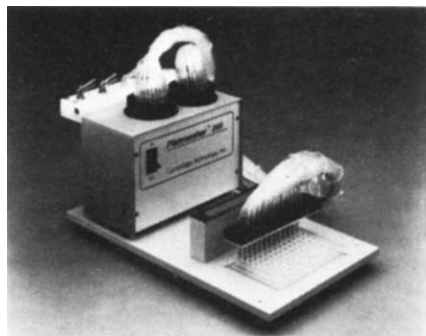
● A recent addition to the Hoefer Scientific range of small gradient makers is a 500-ml model, the SG107S. The capacity of the SG107S makes it suitable for pouring ten or more 14 × 16 cm slab gels collectively in the Hoefer multiple casting chamber, the SE615. The SG107S can also be used for generating buffer gradients in column chromatography. Like the smaller Hoefer gradient makers, this one is made from sturdy acrylic. A simple-to-operate push-pull valve connects two cylindrical chambers which are mounted on a flat acrylic base. A side outlet port at the base of the mixing chamber may be connected to a pump to control the flow rate. Mixing is done on a magnetic stirrer. **Circle No. 110 on Reader Service Card.**



The LKB fraction collector for liquid chromatography.

● LKB-Produkter AB of Sweden has developed a new fraction collector, the 2212 HeliRac, for liquid chromatography. This microprocessor-controlled fraction collector uses circular racks. A wide range of tube sizes can be accommodated and three different collection modes (time, drop and pump signals) are available. Up to six time windows can be programmed for selective collection of peaks with known retention times. HeliRac has a rapid tube change time and is able to synchronize tube change with drop frequency to completely eliminate drops falling between tubes. The instrument case and keyboard are leakproof and resistant to most common solvents. Optional steel racks and a safety tray are available for the collection of aggressive solvents. An optional flow diverter can be used to divert unwanted eluent to a separate container. The collector can be used to control (via an interface) up to three pumps and three valves for automatic buffer changing. **Circle No. 111 on Reader Service Card.**

● The Packard Model 1700 sample processor is a programmable digital diluter with a wide variety of applications. The two modules, diluter and keyboard, perform functions which include pipetting, diluting dispensing and multiple dispensing, sample only transferring, sample aliquoting, serial diluting and titrating. The diluter is driven by a Z-80 microprocessor and can communicate with other instruments via its RS232 port found on the back of the instrument. The keyboard, one 12-button keypad and four command keys, allow the user to communicate with the diluter. A touch-sensitive membrane overlay protects the keyboard from chemical spills. An eight character alphanumeric vacuum fluorescent display shows program information, memory location, and error codes. The movable keyboard module is attached to the sample processor by a flexible cord and can be positioned for the operator's convenience. The instrument will accommodate syringes of various sizes to maximize flexibility. The aspirate syringe pipettes sample or reagent, and the reagent syringe draws in the reagent that is to be dispensed. **Circle No. 112 on Reader Service Card.**



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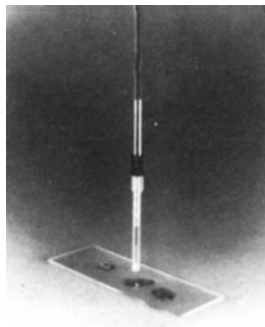
• The Model 260 Platewasher from Cambridge Technology, Inc. of Cambridge, Massachusetts is now available with an optional wash manifold that permits as many as four different wash fluids to be used with the instrument without requiring manual change of tubing connections. The Model 261 manifold is constructed of high density polyethylene. No lubrication is required and the materials are compatible with most common wash fluids such as water, saline, alcohols, and trichloroacetic acid. The Model 260 Platewasher is designed to be used with EIA/ELISA procedures. It is a semi-automated system for washing the wells of microtiter plates. The flow to each of the 24 wash needles is controlled individually and simultaneously, providing high well-to-well uniformity and making the unit much faster than manual techniques. *Circle No. 113 on Reader Service Card.*

• Ultra-pure standards and standard mixtures for performing EPA 600 methods by gas chromatographic analysis are now available from Ultra Scientific. Each kit contains 1ml of each standard required for the particular method plus 1ml of a mixture. Individual standards are supplied in sealed 1ml ampules. Standards are prepared in solution, ready to use and guaranteed to specification. No additional preparation or handling of these hazardous materials is required. Free samples of standard mixtures for any EPA method from 600 to 612 are available on request from Ultra Scientific. *Circle No. 114 on Reader Service Card.*



Ultra's new standards.

• Du Pont's Prep Type OD cartridges for the automated solid phase extraction of lipophilic components from aqueous samples are designed to be used with the Du Pont Prep automated sample processor. The Type OD cartridge partitions components between a moving liquid phase of buffered aqueous sample matrix, wash solvent, or eluting solvent and a solid stationary phase of resin bed. Both aqueous and organic solvents may be used to extract lipophilic analytes from aqueous solutions. Each cartridge comprises a cap, a 4 ml extraction column, a 6 ml effluent cup, and 4 ml recovery cup. Du Pont also provides a method development guide to aid extraction of lipophilic analytes. *Circle No. 115 on Reader Service Card.*



The smallest CO₂ electrode?

• The MI-720 micro-carbon dioxide electrode is claimed to be the smallest commercially available CO₂ electrode capable of measuring CO₂ in small volumes and droplets. Its tip with membrane attached is 3mm in diameter and its total length is 6.2cm. Its useful concentration range is 10⁻² to 10⁻⁴ (440 to 4.4 p.p.m.) CO₂. The MI-720 is one of a range of "small volume" electrodes from Contact Microelectronics, Inc. of Londonderry, New Hampshire. *Circle No. 116 on Reader Service Card.*



From Houston Instruments/Bausch & Lomb, the 4500 MicroScribe.

• The Series 4500 MicroScribe strip chart recorder from Houston Instruments is one of a new generation of recorders using microprocessor control and touch-panel switching. The recorder's centralized liquid crystal display provides constant information about operating parameters as well as self-test messages such as "paper out" or voltage "out of range". Charts from the Series 4500 span a full 20 cm, at speeds ranging from 1 cm per hour to 60 cm per minute. Linearity is within 1% full scale; response is 0.25 seconds for full-scale. *Circle No. 117 on Reader Service Card.*

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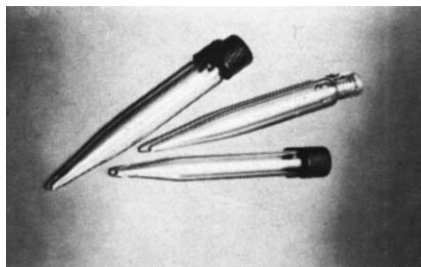
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Kimble's disposable glass centrifuge tubes.

● Kimble disposable borosilicate glass centrifuge tubes combine the advantages of glass and the economy of disposables. Borosilicate glass gives superior chemical durability over plastic, even with pH sensitive solutions and organic solvents. These tubes have standard GPI threads and are available in 5ml, 10ml, and 15ml sizes. Each size fits standard centrifuge cups and can withstand a centrifuge speed of 3,000RCF.

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● A new restriction enzyme, *Asp718*, is now available exclusively from Boehringer Mannheim Biochemicals. This enzyme recognizes the same sequence as *KpnI*, GGATACC, but produces 5' protruding cohesive ends, instead of the 3' ends produced by *KpnI*. This enzyme has one recognition site in simian virus 40 DNA, but no sites in pBR322 or 328 DNA.

Circle No. 119 on Reader Service Card.

● Spectra-Physics' new liquid chromatography sampling system, the SP8780XR automatic sampler, is a stand-alone module which automates any modular liquid chromatography system, while also providing fully integrated operation with other Spectra-Physics LC modules. Control and communication are provided via the company's local area network, LABNET, RS-232-C, and contact closure circuits. A novel feature of the SP8780XR is the ability to use bar code labels on each individual sample vial. The bar codes provide each vial with a unique identification to aid in sample tracking and eliminate errors in tray loading. The code also carries analysis file information for each vial, thereby automating instrument setup in LABNET systems.

Circle No. 120 on Reader Service Card.

● A new bench-top liquid scintillation counter from Beckman, the LS1801, is a three-channel system featuring a rack sample changer that will accommodate either standard vials (336 samples) or miniature vials (648 samples). Beckman's Versa-Rack sample changer allows both types of vial to be used in the same instrument without the need for adapters. An exclusive feature on Beckman counters is the Horrocks number method of quench monitoring. One quench curve generated using *H* number will provide accurate results, regardless of the volume of sample, type or vial or choice of cocktail. The LS1801 can carry out microvolume counting with a sample/cocktail volume as low as 200 μ l, thus cutting consumable costs.

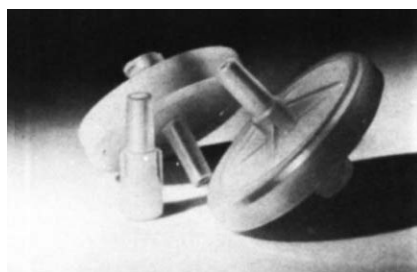
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● Intended for use with the Flow Titertek 8- or 12-nozzle pipettors, the new Ejector Bars produced by Robbins Scientific guarantee ejection of every tip, every time. Made of durable, solid aluminium, they can be attached quickly and easily to Titertek pipettors. They replace existing bars with no pipettor modification required.

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● Normal- and reverse-phase silica media for liquid chromatography have been added to the Amicon Corporation range. Matrex silica chromatographic media are available in a wide range of particle sizes, with pore sizes ranging from 60 to 250 Å. All may be ordered with C18 or C8 functionality for reverse-phase applications. Equivalent capacity and selectivity factors for all particle sizes are especially valuable for direct scale-up to large preparative applications, avoiding the necessity of method redevelopment.

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Spartan filters for use with syringes.

● The Schleicher & Schuell Spartan filtration system, a series of three syringe filters, allows fast, convenient filtration of small volumes when preparing individual HPLC samples. The pre-assembled, disposable syringe filters minimize sample hold-up volumes, save time, and eliminate cross-contamination. The chemically resistant microfilters effectively remove sample and solvent contaminants which can cause erroneous HPLC readings or damage columns, valves or pumps. The filters are designed with a Luer-lock fitting to securely fasten the unit onto the syringe. The Spartan-3 all-nylon filter handles volumes of less than 1 ml with a design that ensures extremely high recovery rates. The S&S nylon-66 membrane of 3-mm diameter is secured in a nylon housing. Suitable for aqueous and most organic filtrations, Spartan-3 can be specified in pore sizes of 0.2, 0.45 or 5.0 μ m. Spartan-25, designed for volumes of 1 to 50 ml, offers the "universal" filtration capability of a nylon-66 membrane in a polypropylene housing. The 25-mm-diameter microfilter completely filters aqueous and most organic samples. Pore sizes are 0.2, 0.45 or 5.0 μ m. Spartan-T is designed for filtering acids or harsh organic solvents that are not compatible with nylon. The chemically resistant, hydrophobic filter unit handles volumes up to 50 ml. Its 0.45- μ m Teflon membrane of 30-mm diameter is housed in a rugged polypropylene holder.

Circle No. 124 on Reader Service Card.

● The 1984 Product Reference Guide from Pharmacia P-L Biochemicals, which lists products for biochemistry and molecular biology, is now available. The guide is divided into sections based on product categories which include: Enzymes, Restriction nucleases, Nucleic acids for molecular biology, Polynucleotides, Nucleotides, Coenzymes, Affinity chromatography, Antibiotics, Buffers, Carbohydrates, Immunologicals, Lectins, Lipids and Phorbol esters.

Circle No. 125 on Reader Service Card.

● New from BBL is an inorganic iodine stain product to improve Gram staining. The new iodine offers an alternative to breakage of glass ampoules to prepare a working iodine solution. The BBL Gram's iodine with concentrate is packaged in component sets containing four 250-ml polypropylene bottles of diluent with four 25-ml bottles of iodine concentrate. As with other BBL Gram stain reagents, the new product features a flip-top cap, compact tray/work station and stain resistant labels. The two bottles mix readily to prepare the working iodine solution. The current BBL stabilized PVP iodine products (in kits, sets or gallon form) are still available to microbiologists who prefer the elimination of the working solution preparation step and a longer stability.

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● Oligonucleotide hybridization probes of extremely high specific activity have been used successfully to detect many different genes by hybridization analysis. A new technical information bulletin (Bulletin T-1588) from Beckman Instruments, Inc. shows how to use the Model 170 radioisotope detector to automate the sample handling of the preparation of labelled oligonucleotides to minimize radiation exposure. The detector can be used for desalting labelled oligonucleotides, minimizing the exposure to large amounts of radiation and providing analog records of the run in addition to integration data that enables easy calculation of the specific activity.

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● A new addition to Vector Laboratories biotin/avidin system line of products is the amplifying agent biotinylated anti-avidin D. This molecule is capable of binding to avidin D via its biotin residues as well as through conventional antibody-antigen interactions. The potential for multiple-site binding appears to provide this antibody with unusual characteristics. When used in conjunction with fluorescent or enzyme-conjugated avidin D, a large amplification of the primary signal can be achieved. This reagent can be used with biotinylated DNA hybridization probes, hormones, toxins, antibodies or lectins to enhance the desired signal.

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These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

Awards

Twenty-nine research fellowships have been awarded under the European Science Exchange Programme as follows: **Ms Victoria J. Allan**, Department of Biology, University of York, to spend one year from February 1985 at the European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; **Mr Ilesh Bidd**, School of Chemistry, University of Bristol, to spend one year from January 1985 at the Institut de Chimie, Université Louis Pasteur de Strasbourg, France; **Mr Alexander W. Blayney**, University Hospital of Wales, Cardiff, to spend one year from January 1985 at the department of otolaryngology, University of Bordeaux, France; **Mr David H. Burgess**, School of Mathematical Sciences, Queen Mary College, London, to spend one year from January 1985 at the section d'astrophysique, Observatoire de Paris, Meudon, France; **Mr I. Colin G. Campbell**, Department of Mathematics, King's College, London, to spend one year from January 1985 at the Institute of Theoretical Physics, University of Stockholm, Sweden; **Mr David B. Carpenter**, Department of Physics, University of Edinburgh, to spend nine months from January 1985 at the Deutsches Elektronen-Synchrotron, Hamburg, Federal Republic of Germany; **Mr Christopher Carr**, Department of Inorganic Chemistry, University of Bristol, to spend one year from January 1985 at the Department of Inorganic Chemistry, Royal Institute of Technology, Stockholm, Sweden; **Mr Christopher L. Clayton**, Department of Applied Biology, University of Wales Institute of Science and Technology (UWIST), Cardiff, to spend one year from January 1985 at the Department of Medical Biochemistry, University of Geneva, Switzerland; **Miss Margaret E. Collins**, Department of Botany and Microbiology, University College, Cardiff, to spend one year from January 1985 at the Institut de Génétique Moléculaire, Centre National de la Recherche Scientifique, Gif-sur-Yvette, France; **Mr George M. Coupland**, Department of Molecular Biology, University of Edinburgh to spend one year from January 1985 at the Institut für Genetik, University of Cologne, Federal Republic of Germany; **Mr Irwin B. Davidson**, MRC Institute of Virology, University of Glasgow, to spend one year from March 1985 at the Laboratoire de Génétique moléculaire des Eucaryotes, Centre National de la Recherche Scientifique, Strasbourg, France; **Mr Christopher S. Durbin**, School of Geography, University of Manchester, to spend one year from July or September 1985 at the Laboratoire de Géomorphologie, Université Libre de Bruxelles, Belgium; **Mr John L. Edwards**, School of Chemistry, University of Bristol, to spend one year from January 1985 at the

Collège de France, Paris, Francis; **Dr Catherine W. Gill**, Marine Science Laboratories, University College of North Wales, Menai Bridge, to spend one year from October 1984 at the Station Marine de Roscoff, France; **Mr David R. Greaves**, Biophysics Department, King's College, London, to spend one year from January 1985 at the Netherlands Cancer Institute, Amsterdam, the Netherlands; **Miss Kim E. Hammond**, School of Biological Sciences, University of East Anglia, Norwich, to spend one year from January 1985 at the department of phytopathology, Agricultural University, Wageningen, the Netherlands; **Dr Barbara J. Hart**, Department of Zoology, University of Glasgow, to spend one year from September 1984 at the Institut Royal des Sciences Naturelles de Belgique, Brussels; **Miss Elaine J. Hughson**, Department of Haematology, Royal Hallamshire Hospital, Sheffield, to spend one year from October 1984 at the European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; **Mr Benjamin P. Jeffries**, Mathematical Institute, University of Oxford, to spend one year from January 1985 at the Max Planck Institute for Physics and Astrophysics, Munich, Federal Republic of Germany; **Dr Deborah J. Jones**, Department of Chemistry, University of Southampton, to spend one year from 1 October 1984 at the Laboratoire des Acides Minéraux, Université des Sciences et Technique du Languedoc, Montpellier, France; **Dr Peter J. McDonald**, Department of Physics, University of Nottingham, to spend one year from January 1985 at the Service de Physique du Solide et de Résonance Magnétique, Saclay, France; **Dr Janice G. Mather**, Department of Zoology, University of Manchester, to spend one year from October 1984 at the Institute of Zoology and Zoophysiology, University of Aarhus, Denmark; **Mr Ronald A. Milligan**, Department of Structural Biology, Stanford University School of Medicine, California, USA, to spend one year from April 1985 at the European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany; **Dr Jonathan Mitchley**, Botany School, University of Cambridge, to spend one year from January 1985 at the Department of Plant Ecology, University of Utrecht, the Netherlands; **Mr Michael J. Morris**, School of Chemistry, University of Bristol, to spend one year from January 1985 at the Institut de Chimie, Université Louis Pasteur, Strasbourg, France; **Mr Mark A. Riley**, Department of Physics, University of Liverpool, to spend one year from October 1984 at the Niels Bohr Institute, University of Copenhagen, Denmark; **Mr Andrew Robinson**, Department of Astronomy, University of Manchester, to spend one year from January 1985 at the European Southern Observatory, Garching bei München, Federal Republic

of Germany; **Mr Robin G. Stuart**, Clarendon Laboratory, University of Oxford, to spend one year from May 1985 at the theory division, CERN, Geneva, Switzerland; **Dr Simon G. Webster**, School of Animal Biology, University College of North Wales, Bangor, to spend one year from October 1984 at the Institut für Zoophysiology, Rheinische Friedrich-Wilhelms-Universität, Bonn, Federal Republic of Germany.

The Royal Society of Chemistry has announced a cycle of new awards for British and Commonwealth citizens sponsored by various industrial companies; separate prizes are available to scientists working in the fields of: Analytical Separation Methods; Chemical Analysis and Instrumentation; Chemical Education; Health Safety or Environmental Chemistry; Hydrocarbon Oxidation Chemistry; Main Group Element Chemistry; Natural Product Chemistry; Organic Reaction Mechanisms; Solids and Materials Processing; Spectroscopy; Structural Chemistry; and Thermodynamics. Awards will be available for a different range of subjects in subsequent years. Further details from Mrs Y.A. Fish, The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK.

The first in a series of scientific research funding awards, worth over £380,000 a year has been announced by the Wellcome Trust, Britain's largest medical charity, in association with the Royal Australasian College of Physicians. The first two Australian Wellcome Senior Research Fellows will be **Dr. David Healy** of the Prince Henry's Hospital, Melbourne, who will work on hormone activity in pregnancy during his studies on endocrinology in obstetrics and gynaecology, and **Dr Keryn Williams** who will be investigating the problems of rejection in corneal transplants at Flinders University, Adelaide.

To celebrate one hundred years of publication of Baillière's Clinical Pharmacology, **Baillière Tindall** is sponsoring an essay competition for medical and pharmacy undergraduates. There is a first prize of \$1,000 and two second prizes of \$500; further information is available from The Editorial Director, Baillière Tindall, 1 St Anne's Road, Eastbourne, East Sussex BN21 3UN, UK. The Royal Swedish Academy of Sciences has awarded the **Crafoord Prize** for ecological research to **Professor Danieal H. Janzen**, a Professor at the University of Pennsylvania, USA, for his studies on co-evolution.

A major European Conservation Award scheme has been announced by the **Ford Motor Company**, operating in eight European countries: Austria, Holland, Belgium, Italy, Spain, Switzerland, France, and the United Kingdom. Total funds exceed \$100,000 and projects can be submitted in up to six different categories

including rural, urban and heritage conservation. Further information can be obtained from The Conservation Foundation, 11a West Halkin Street, London, SW1X 8JL, UK.

The International Agency for Research on Cancer is inviting applications for its Training Fellowships and a Visiting Scientist Award for 1985/86. Further details obtainable from the Chairman of the Fellowships Selection Committee, International Agency for Research on Cancer, 150 Courts Albert-Thomas, 69372 Lyon Cedex 08, France.

Pool Bioanalysis Italiana, is inviting entries of scientific papers on environmental microbiology in their first international competition; the prize is one week's holiday in Rome. Any paper in which SAS, SAS Compact or MTM-3 air samplers have been used is acceptable. For further details write to "P.B.I.", 1st International Competition, Via Novara 89, 20153 Milano, Italy.

Nominations are now open for the 1985 **Lita Annenberg Hazen Awards for Excellence in Clinical Research**, the purpose of which is to encourage increased participation in clinical research by physicians. Prizes amounting to \$100,000 are available and further details can be obtained from James F. Glenn, M.D., President, The Mount Sinai Medical Centre, Chairman, The Lita Annenberg Hazen Awards Program, 1 Gustave L. Levy Place, New York, New York 10029, USA.

The **Smithsonian Institution** award post-doctoral, predoctoral and graduate research fellowships in the fields of *inter alia*, the History of Science and Technology, Earth Sciences, Anthropology, and Biological Sciences. For details and application forms contact the Office of Fellowships and Grants, Desk J, 3300 L'Enfant Plaza, Smithsonian Institution, Washington, D.C. 20560, USA.

Appointments

Dr Denver Hall has been appointed to the Unilever Chair in Colloid and Surface Chemistry at the University of Salford.

Professor Barry R. Jennings of the University of Brunel has been appointed as the new Professor of Physics at the University of Reading.

Meetings

14 November, **Meeting on the Use of Physical and Analytical Techniques in Drugs Research**, London (Conference Secretariat, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS, UK).

22-23 November, **Clonal Evolution of Tumours**, British Association for Cancer Research/Royal Society of Medicine Joint Winter Meeting, London (Barbara Cavilla,

BACR, c/o Institute of Biology, 20 Queensberry Place, London SW7 2DZ, UK).

29 November, **One Day Symposium on Food Production and Our Rural Environment — The Way Ahead**, London (Centre for Agricultural Strategy, University of Reading, 1 Early Gate, Reading RG6 2AT, UK).

4-6 December, **8th International Online Information Meeting**, London (Learned Information (Europe) Ltd, Besselsleigh Road, Abingdon, Oxford OX13 6LG, UK).

25 February-1 March, **36th Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy**, New Orleans, USA (Mr David R. Weill III, 437 Donald Road, Pittsburgh, Pennsylvania 15235, USA).

25 February-1 March, **International Conference on Climatic, Biotic and Human Interactions in the Humid Tropics**, Sao Jose dos Campos, Brazil (Mr Walter Shearer, Senior Programme Officer, Development Studies Division, The United Nations University, Toho Seimei Building, 15-1, Shibuya 2-chome, Shibuya-ku, Tokyo 150, Japan).

25-28 March, **2nd Conference of the International Association for Breast Cancer Research**, London (Dr Marvin Rich, AMC, Cancer Research Center, 6401 West Colfax Avenue, Lakewood, Colorado 80214, USA).

1-4 April, **3rd International Meeting on Low Temperature Microscopy and Biological Analysis** Cambridge, UK (The Administrator, Royal Microscopical Society, 37/38 St. Clements, Oxford OX4 1AJ, UK).

3-4 April, **21st Annual Meeting of the National Council on Radiation Protection and Measurements**, Washington D.C., USA (Mr Roger Ney, NRC, 7910 Woodmont Avenue, Suite 1016, Bethesda, Maryland 20814, USA).

10-12 April, **International Symposium on Chemistry and Physics of Baking: Materials, Processes and Products**, Sutton Bonington, Leicestershire, UK (Mr J.M.V. Blanshard, Department of Applied Biochemistry and Food Science, The University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, LE12 5RD, UK).

16-18 April, **International Symposium on the Impact of Biotechnology on Diagnostics**, Rome, Italy (Organizing Secretariat, Fondazione Giovanni Lorenzi, Via Montenapoleone 23, Milan, Italy).

16-18 April, **Conference on Advanced Computational Techniques for Information Retrieval**, Oxford, UK (The Association for Information Management, 3 Belgrave Square, London SW1X 8PL, UK).

23-24 April, **Meeting on Material Properties and Stress Analysis in Biomechanics**, Brunel University, UK (The Institute of Physics, Meetings Office, 47 Belgrave Square, London SW1 8QX, UK).

27 April-2 May, **Congress on Laboratory Medicine**, Hamburg, FRG (Dr O. Fenner, Organizing Committee, Kongress für Laboratoriumsmedizin, Hamburg Messe und Congress GBH, Ausstellungsabteilung, Postfach 30 24 80, D-2000 Hamburg 36, FRG).

28 April-1 May, **International Conference on Arctic Water Pollution Research: Applications of Science and Technology**, Yellowknife, Northwest Territories, Canada (Conference Services Office, National Research Council of Canada, Montreal Road Laboratories, Ottawa K1A 0R6, Canada).

28 April-4 May, **21st EUCHEM Conference on Stereochemistry**, Bürgenstock, Switzerland (Professor M. Julia, Laboratoire de Chimie, E.N.S., 24 rue l'homond, 75231 Paris Cedex-05, France).

29 April-2 May, **23rd International Magnetics Conference**, St. Paul, Minnesota, USA (Mr J.A. Nyenhuis, School of Electrical Engineering, Purdue University, West Lafayette, Indiana 47907, USA).

30 April-3 May, **Conference on Computer Aided Production Management**, Wembley, Middlesex, UK (The Manager — Conferences and Exhibitions, The Institution of Production Engineers, Rochester House, 66 Little Ealing Lane, London W5 4XX, UK).

7 May, **37th International Symposium on Crop Protection**, Ghent, Belgium (The Secretary, Faculteit Van de Landbouwwetenschappen, Rijksuniversiteit, Coupure Links, B-9000 Gent, Belgium).

9-10 May, **International Symposium on Thyroid Function Tests**, Ile de Bendor, France (The Secretariat, Laboratoire des Hormones Proteiques, Faculté de Médecine, F-13385 Marseille Cedex 5, France).

21-23 May, **Biotech 85 Europe**, Geneva, Switzerland (Online Conferences Ltd, Pinner Green House, Pinner, Middlesex, HA5 2AE, UK).

21-23 May, **7th Annual Symposium on Safeguards and Nuclear Material Management** Liège, Belgium (Mr L. Stanchi, CEE-JRC, I-21020 Ispra Varese, Italy).

22-24 May, **International Congress on Tissue Integration in Oral and Maxillo-Facial Reconstruction**, Brussels, Belgium (Dr D. van Steenberghe, Faculty of Medicine, Catholic University Leuven, Kapucijnenvoer 7, B-3000 Leuven, Belgium).

3-7 June, **International Symposium on Vaccines and Vaccinations**, Paris, France (Secretariat, Symposium on Vaccine and Vaccinations 1985, Institut Pasteur, 28 Rue du Docteur Roux — 75724 Paris Cedex 15, France).

4-6 June, **International Seminar on Electronics and Traffic on Major Roads**, Paris, France (Secretariat of the Seminar on Electronics and Traffic, European Conference of Ministers of Transport, 19 rue de Franqueville, 75775 Paris Cedex 16, France).